PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:	WO 99/37331
A61K 48/00, C07H 21/04, C12N 15/63, 15/86, 5/10, 15/40	A1	(43) International Publication Date:	29 July 1999 (29.07.99)

(21) International Application Number: PCT/US99/01382

(22) International Filing Date: 21 January 1999 (21.01.99)

(30) Priority Data:
60/072,033 21 January 1998 (21.01.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/072,033 (CON)
Filed on 21 January 1998 (21.01.98)

(71) Applicant (*for all designated States except US*): THE REGENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; Technology Management Office, Wolverine Tower, Room 2071, 3003 South State Street, Ann Arbor, MI 48109–1280 (US).

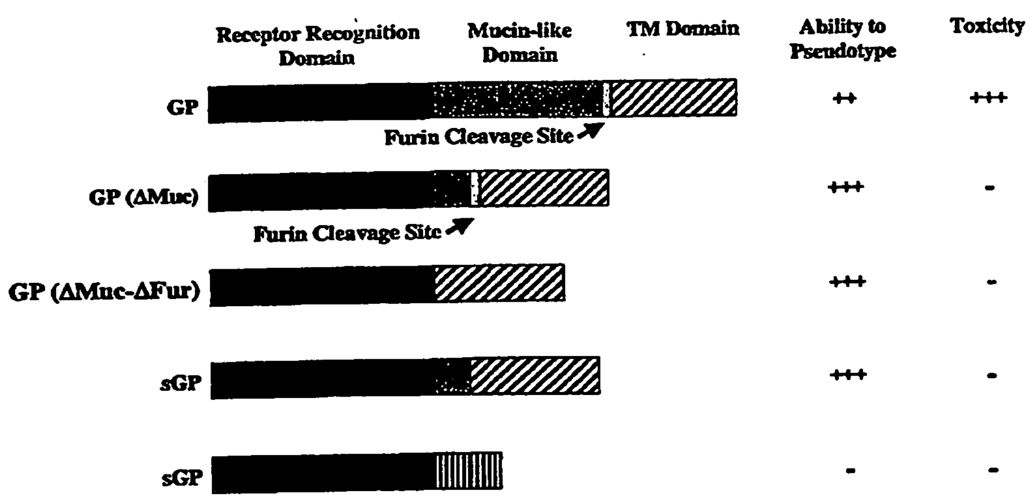
(72) Inventors; and
(75) Inventors/Applicants (*for US only*): NABEL, Gary, J. [US/US]; 385 Meadow Creek Drive, Ann Arbor, MI 48105 (US). DELGADO, Rafael [ES/US]; 1265 Crescent, Ann Arbor, MI 48103 (US). YANG, Zhi–yong [CN/US]; 2863 Leslie Park Circle, Ann Arbor, MI 48105 (US).

(74) Agents: SMITH, DeAnn, F. et al.; Harness, Dickey & Pierce, P.L.C., P.O.Box 828, Bloomfield Hills, MI 48303 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: TARGETING GENE TRANSFER VECTORS TO CERTAIN CELL TYPES BY PSEUDOTYPING WITH VIRAL GLYCOPROTEIN



(57) Abstract

The present invention provides compositions and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein, such as that from Ebola virus. The methods comprise the step of administering to a cell population a gene to be transferred operatively linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of viral glycoprotein.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TARGETING GENE TRANSFER VECTORS TO CERTAIN CELL TYPES BY PSEUDOTYPING WITH VIRAL GLYCOPROTEIN

FIELD OF THE INVENTION

The present invention relates generally to compositions and methods for selective gene transfer, and in particular, to methods for targeting genes to certain cell types, comprising introducing to a cell population the gene to be transferred operatively-linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of viral glycoprotein.

BACKGROUND OF THE INVENTION

Ebola virus has been identified as the cause of several highly lethal outbreaks of hemorrhagic fever. Infection begins typically with flu-like symptoms which often progress rapidly to fatal complications of hemorrhage, fever, and hypotensive shock. Bowen, E.T.W. et al., *Lancet* 1:571 (1977); Centers for Disease Control, *M.M.W.R.* 44:381 (1995); Le Guenno, B. et al., *Lancet* 345:1271 (1995); Peters, C.J. et al., *Fields Virology*, B.N. Fields, D.M. Knipe and P.M. Howley, Eds. (Lippincott-Raven, Philadelphia) p. 1161 (1996). The negative-stranded genome of Ebola virus contains seven structural and regulatory proteins (Sanchez, A. et al., *Virus Res.* 29:215 (1993)), but despite its relative simplicity, the molecular basis for Ebola virus pathogenicity is unknown. Among the viral gene products, the glycoprotein is found in two forms: a secreted form, 50-70 kD (Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996)), synthesized at high levels early in the course of infection, and an alternative transmembrane form, which arises from RNA editing to encode a 120-150 kD glycoprotein that is incorporated into the virion. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996); Volchkov, V.E. et al., *Virology* 214:421 (1995). The first 295 amino acids (aa) of both proteins are identical in the Zaire strain, while sGP contains an additional 69 and GP another 381 COOH- terminal aa residues. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996). The specific cellular targets of these related gene products and their roles in the pathogenesis of Ebola infection have not been characterized.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein. In one embodiment, the methods of the invention comprise the

step of administering to a cell population a gene to be transferred operatively-linked to an appropriate transfer vehicle, wherein the transfer vehicle is associated with a transmembrane form of Ebola glycoprotein. In this embodiment, the gene will be targeted to cell types naturally infected with Ebola such as endothelial cells, monocytes and hepatocytes.

Genetic constructs for selective gene transfer into certain cell types are also provided. The genetic constructs of the present invention comprise a gene to be transferred operatively-linked to an appropriate transfer vehicle or carrier, wherein the transfer vehicle or carrier is associated with a transmembrane form of viral glycoprotein. In one embodiment, the transmembrane form of Ebola glycoprotein is expressed on the surface of a virus-based gene-targeting vector, *e.g.*, lentiviral or retroviral vector. In another embodiment, an expressed or synthesized transmembrane glycoprotein is chemically derivatized to a non-biologic gene targeting vehicle.

Additional objects, advantages, and features of the present invention will become apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings.

Figures 1A-1C show the binding of sGP to neutrophils;

Figures 2A-2D show the infection of different cell types by a GP-pseudotyped vector of the present invention;

Figures 3A-3F show the dependence of sGP binding on CD16b and correlation of binding with neutrophil activation;

Figures 4A-4B show the effect of sGP on neutrophil function;

Figures 5A-5C show the infection rate of cells with a GP-pseudotyped retroviral vector of the present invention;

Figure 6 is a schematic of the plasmid pVR 1012-GP(IC) (Ivory Coast strain of GP, see SEQ ID NO: 1);

Figure 7 is a schematic of the plasmid pVR 1012-GP(S) (Sudan strain of GP, see SEQ ID NO: 2);

Figure 8 is a schematic of the plasmid pVR 1012-GP(Z) (Zaire strain of GP, see SEQ ID NO: 3);

Figure 9 is a schematic of the plasmid pVR 1012-sGP(Z) (Zaire strain of sGP, see SEQ ID NO: 4); and

Figure 10 is a summary of the characterization of GP and sGP derivatives for their ability to pseudotype to induce cytotoxicity in producer cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides genetic constructs and methods for targeting gene transfer vectors to certain cell types by pseudotyping with a transmembrane form of viral glycoprotein. The methods for selective gene transfer of the present invention comprise the step of administering to a cell population a genetic construct of the present invention so that the gene is transferred and expressed in certain cell types present in the cell population. Administration to the cell population may be *ex vivo* or *in vivo*.

The genetic constructs of the present invention comprise a gene to be transferred operatively-linked to an appropriate transfer vehicle or carrier, wherein the transfer vehicle or carrier is associated with a transmembrane form of viral glycoprotein. In one embodiment, the transmembrane form of Ebola glycoprotein is associated with the vehicle or carrier. The gene to be transferred will thus be targeted to cell types naturally infected with Ebola virus including endothelial cells, hepatocytes, monocytes and related cell types such as dendritic cells. The transmembrane form of Ebola glycoprotein may be chosen from, without limitation, the Ivory Coast strain (SEQ ID NO: 1), Sudan strain (SEQ ID NO: 2), the Zaire strain (SEQ ID NO: 3) and/or the Reston strain. It will be appreciated that in other embodiments of the present invention, other hemorrhagic fever virus glycoproteins, in particular transmembrane glycoproteins, may be employed and will target those cell types naturally infected by the virus. Examples of hemorrhagic viruses include dengue virus, Yellow Fever virus (*flaviviridae*); Lassa, Junin and Machupo (*arenaviridae*); Rift Valley, Congo-Crimean and Hantaan (*bunyaviridae*); and Marburg (*filoviridae*). It will also be appreciated that derivatives of the transmembrane glycoprotein which retain the capability of targeting specific cell types, may also be employed, for example, the transmembrane glycoproteins may be mutated, *e.g.*, toxic regions may be removed to improve producer cell viability (see Figure 10).

The transmembrane glycoprotein may be expressed on the surface of a virus-based gene-targeting vector, *e.g.*, lentiviral, retroviral, replication-deficient retroviral, adenoviral or adeno-associated viral vector. The transmembrane glycoprotein may

also be expressed or synthesized and chemically derivatized to a non-biologic gene targeting vehicle, *e.g.*, liposome or DNA-protein complex.

The term "operatively-linked" as used herein refers to functional linkage between a nucleic acid expression control sequence (such as a promoter) and a second nucleic acid sequence (*i.e.*, gene), wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence. Expression control sequences are known to those skilled in the art (see, *e.g.*, Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990)). "Associated with" as used herein refers to the transmembrane form of viral glycoprotein being in contact or linkage with the transfer vehicle or carrier in such a way as to direct the transfer vehicle or carrier to certain cell types. The terms "transfer vehicle" and "carrier" refer to any type of structure which is capable of delivering the gene of interest to a target cell.

Many transfer vehicles or carriers are known in the art. For example, various viruses that are capable of infecting cells can be recombinantly manipulated to carry the gene of interest without affecting their infectivity. As used herein, the terms "infect" and "infectivity" refer only to the ability of a virus to transfer genetic material to a target cell. Those terms do not mean that the virus is capable of replication in the target cell. In fact, it is preferable that such viruses are replication defective so that target cells do not suffer the effects of viral replication.

In one embodiment, the virus employed is a replication defective retroviruses. When these replication defective retroviruses are employed, their genomes can be packaged by a helper virus in accordance with well-known techniques. Suitable retroviruses include PLJ, pZip, pWe and pEM, each of which is well known in the art. Suitable helper viruses for packaging genomes include ψCrip, ψCre, ψ2, ψAm and adeno-associated viruses.

In another embodiment, lentiviral vectors are employed. Surprisingly, the inventors of the present invention were successful in pseudotyping lentiviral vectors (HIV) with the transmembrane glycoprotein from Ebola. Feline immunodeficiency virus, bovine immunodeficiency virus, simian immunodeficiency virus and EAIV, may also be employed as the carrier in the compositions and methods of the present invention.

Gene delivery systems other than viruses may also be employed. For example, the gene to be transferred may be packaged in a liposome which is chemically derivatized to the transmembrane glycoprotein. To form these liposomes,

one mixes the DNA of an expression vector which expresses the gene to be transferred with lipid, such as *N*-[1-(2,3,-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) in a suitable buffer, such as Hepes buffered saline. This causes the spontaneous formation of lipid-DNA complexes (liposomes). Felgner, P.L. et al., *PNAS (USA)* 84:7413-7417 (1987).

Another gene delivery system that may be utilized in this invention is DNA-protein complexes. The formation of DNA-protein complexes is described in United States Patent No. 5,166,320, the disclosure of which is herein incorporated by reference.

It will be appreciated that any gene may be employed in the compositions and methods of the present invention. For example, and without limitation, in the treatment of cancer, death inducing genes, including genes coding for cytostatic or cytotoxic proteins, *e.g.*, HSV tk, and genes encoding cyclin dependent kinase inhibitors, p21, p27, cytosine deaminase, and fas ligand, may all be employed. In another example, for the treatment of cardiovascular or ischemic vascular disease, genes encoding angiogenic factors such as VEGF basic or acidic FGF's (FGF 1-5) may be employed. In yet another example, in the treatment of vasospasm, the gene encoding NO synthase or heme oxygenase, may be employed. In a further example, monocytes and dendritic cells may be targeted with genes encoding immunogens for cell-targeted immunization.

In one embodiment, the methods of targeting gene transfer vectors to certain cell types involve administering to a cell population *ex vivo*, a construct of the present invention and introducing the transfected cells into a subject. In an alternative embodiment, the methods of the present invention comprise administering to an *in vivo* cell population a construct of the present invention. Administration can be by any of the routes normally used for *in vivo* gene therapy such as direct delivery to cells via a gene gun, and other known techniques. The constructs are thus administered in any suitable manner, preferably with pharmaceutically acceptable carriers. The constructs can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. The preferred method of administration will often be intravenous.

To practice an *ex vivo* method of the present invention, a source of cells is obtained. The cells are optionally selected from *in vitro* cells, such as those derived from cell culture and *ex vivo* cells, such as those derived from a subject. The term "subject" is intended to include living organisms, *e.g.*, mammals. Examples of

subjects include humans, primates, dogs, cats, mice, rats, and transgenic species thereof. It will be appreciated that specific cell populations may be obtained by isolation from certain tissues by methods known to those skilled in the art. The cells are maintained under conditions necessary to support growth, for example an appropriate temperature (*e.g.*, 37°C) and atmosphere (*e.g.*, air plus 5% CO_2).

The cells are then transfected with the constructs of the present invention by introducing the constructs to the cell population, under conditions favorable for transfection. According to one embodiment of the present invention, cells are treated with compounds that facilitate uptake of the constructs by the cells. According to another embodiment of the present invention, cells are treated with compounds that stimulate cell division and facilitate uptake of the constructs. It will be appreciated that compounds that facilitate uptake of constructs by cells and compounds that stimulate cell division are known to those skilled in the art.

The constructs of the present invention express the transferred gene in a dose dependent manner. The specific dose to be administered to a patient will be determined by the efficacy of the particular construct and/or delivery system employed, the gene transferred, and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular construct or effect a particular patient. In determining the effective amount of the construct or transfected cell to be administered, the physician needs to evaluate circulating plasma levels, toxicities, and progression of disease. It will be appreciated that administration can be accomplished via single or divided doses.

There is a wide variety of suitable formulations for pharmaceutical compositions containing the constructs of the present invention. Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the construct dissolved in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the construct, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. The construct, alone or in combination with other suitable components, may also be made into aerosol formulations to be administered via inhalation, *e.g.*, to the bronchial passageways. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the construct with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the construct with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules or vials. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transfected by the constructs as described above in the context of *ex vivo* therapy can also be administered as described above.

This invention also provides compositions and kits comprising the constructs of the present invention. For example, the composition can comprise the constructs of the present invention in a pharmaceutically acceptable carrier as described above. Kits comprising such compositions and instructions for use are also within the scope of this invention.

In order to more fully demonstrate the advantages arising from the present invention, the following examples are set forth. It is to be understood that the following is by way of example only and is not intended as a limitation on the scope of the invention.

SPECIFIC EXAMPLE 1

I. Methods

Recombinant retroviruses were produced by transient transfection of 293T cells: 2 x 10^6 cells were plated 24 hours before transfection in 60 mm dishes. Transfection was performed by calcium-phosphate precipitation using 3 μg of a retroviral vector (Kinsella, T.M. et al., *Hum. Gene Ther.* 7:1405 (1996)) encoding lucerifase linked to an internal ribosome entry site and a green fluorescent protein derivative (GFP; pEGFP, Clontech), pLZRs-Luc-Gfp, 5 μg of an expression vector

encoding gag and pol, pNGVL-MLVgag-pol, and 1 μg of the envelope encoding plasmid: pNGVL-4070A (ampho) env, pCMV-Eco env or p1012-Ebola GP, respectively. Supernatants corresponding to 24-48 hours post-transfection were harvested, cleared by low-speed centrifugation and either used immediately for infection or frozen at -80°C. Infections were performed in 6-well plates (1.5-2.5 x 10^5 adherent cells) or 12-well plates (5 x 10^5 non-adherent) using different dilutions of the supernatants by incubating the cells overnight with 1 ml and 300 μl, respectively of the diluted supernatants. Polybrene was used at a concentration of 5 μg/ml for all the cell lines except for D17 in which the concentration was 100 μg/ml. After overnight infection, fresh medium was added and the cells were incubated for an additional 24 hours. After infection, the cells were lysed in 25 mM Tris-phosphate pH 8, 2 mM DTT, 2 mM 1,2-diaminocyclohene-N,N,N',N'-tetraacetic acid, 10% glycerol, 1% TritonX-100, and assayed for luciferase activity using Luciferase Assay Reagent (Promega, Madison, WI) in a 1251 BioOrbit Luminometer. The same number of cells (range 5-10 x 10^4) was analyzed for every specific cell line.

Binding of sGP to neutrophils and inverse correlation of binding with activation: *Figures 1A-1A2.* PBMC from normal volunteers were incubated with control or sGP supernatants derived from transfected 293 cells, and immunostaining was performed using a rabbit antibody to sGP as previously described. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996); Xu, L. et al., *Nat. Med.* (1997) in press. Secondary staining was performed with a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Sigma, F9887). All incubations were performed at 4°C for 30 minutes with .4 μg of the relevant antibodies per 10^6 cells in a 50 μl volume.

Figures 1B-1B1. Double immunostaining with antibodies to sGP and the neutrophil-specific marker, CD15. Cells were incubated with a FITC conjugated mouse anti-human CD15 antibody (Caltag, cat# MHCD1501), followed by secondary staining with a PE-conjugated anti-rabbit IgG antibody (Sigma) to detect sGP binding. Cells were washed with PBS, fixed in 1% formaldehyde, and analyzed by FACS.

Figure 1C. Specific absorption of sGP by neutrophils. Control or sGP supernatants derived from relevant transfected 293 cells (Xu, L. et al., *Nat. Med.* (1997) in press) were incubated at 1:500 dilution with 10^6 mononuclear or granulocytic cells. Cells were removed and the resulting supernatants analyzed by an 8% SDS PAGE gel. Western blot analysis was performed as previously described (Xu, L. et al., *Nat. Med.* (1997) in press) using an anti-GP rabbit antisera and a secondary

antibody, horseradish peroxidase conjugated donkey anti-rabbit IgG at a dilution of 1:5,000 (Amersham, NA934). Primary antibody was incubated for 30 minutes at room temperature, as was the secondary antibody. The immunocomplexes were detected by chemiluminescence using Supersignal® chemiluminescent substrate reagents (Pierce) according to the manufacturer's instructions. Arrow indicates sGP reactive band.

Infection of different cell types by GP-pseudotyped retroviral vector and preferential binding to endothelial cells: *Figure 2A.* Infection of different indicator cell lines with the Ebola-GP pseudotyped retrovirus expressing luciferase. Amphotropic and ecotropic retroviral vectors were used as controls. Viruses were diluted to different multiplicities of infection (MOI) to provide for equal luciferase activity on Hela cervical epithelial cells, permissive for amphotropic retrovirus, D17 dog osteosarcoma cells (Embretson, J.E. et al., *J. Virol.* 61:3454 (1987)), which are permissive for amphotropic, xenotropic, and ecotropic retroviruses, and BW5147 T leukemia cells permissive for amphotropic and ecotropic virus. In these groups, GP virus titer was 1-4x10^5/ml and amphotropic virus was ~2x10^4/ml (MOI's $\approx$1.0 and 0.1, respectively), and the ecotropic virus titer was ~10^6/ml (MOI$\approx$10). Titers were determined by endpoint dilution of reporter activity of the amphotropic virus in D17 cells, and was normalized to reverse transcriptase activity for the GP virus.

Figure 2B. Analysis of different normal or transformed cell lines by infection with amphotropic or GP retroviral vectors at the same titer (10^4/ml, MOI$\approx$0.2). Forty-eight hours after infection, an equivalent of 5 x 10^4 cells was assayed for luciferase activity after exposure to equal titers of viral stocks. Luminescence is expressed as the fold-increase over non-infected control cells.

Figures 2C-2C3. The binding of sGP (left) or GP-pseudotyped retrovirus (right) to neutrophils (upper panel) or microvascular endothelium (lower panel) was determined by FACS. sGP binding was performed as in Fig. 1A, and retrovirus incubation was performed at 37°C for 2 hours in the presence of polybrene (8 μg/ml).

Figure 2D. Infection of D17 cells by GP-pseudotyped virus in the absence (lane 1, none) or presence of control (lane 2) or sGP supernatant (lane 3) from transfected 293 cells. Gene transfer was measured by the luciferase assay as described below. Luminescence refers to relative light units in the luciferase assay.

Depend nce of sGP binding n CD16b and correlation of binding with neutrophil activation: *Figures 3A-3D.* Neutrophils were incubated for 30 minutes at 4°C with a mouse antibody to CD16b (upper panel; clone 3G8 from Immunotech,

cat# 1M0813) or CD62L (middle panel, R&D Systems), compared to the indicated control antibody [purified mouse IgG (Vector Laboratories), I-2000], followed by supernatants from control or sGP-transfected 293 cells, primary rabbit antibody to sGP, and a FITC-conjugated secondary antibody to rabbit IgG (Fig. 1, legend). Cells were washed with PBS, fixed in 1% formaldehyde, and analyzed by FACS. For blocking, 10^6 cells were incubated with 0.5 – 1 μg of the relevant antibodies for 30 minutes in a 50 μl volume.

Figures 3E-3F. Immunostaining with sGP was performed on isolated neutrophils which were maintained in media (none) or incubated with PMA (10 ng/ml) at 37°C for 30 minutes (PMA).

Effect of sGP on neutrophil function: *Figures 4A-4B.* Exposure of neutrophils to sGP inhibits down modulation of L-selectin. Isolated neutrophils were incubated with the indicated control or sGP containing supernatants (Xu, L. et al., *Nat. Med.* (1997) in press) and defined media (AIM V, GIBCO) for 4 hours at 37°C. Expression of L-selectin was determined using an anti-CD62L antibody (R&D Systems), followed by the secondary staining using a FITC-conjugated anti-mouse IgG (Sigma, F2883) as described in Fig. 1, legend. Cells were washed with PBS, fixed with 1% formaldehyde and analyzed by FACS for relative levels of fluorescence intensity as a function of cell number. An isotype control was used to quantitate background levels of immunostaining (neg.). Results are representative of three independent experiments.

II. Results

To determine the specificity of Ebola virus glycoproteins, expression vectors encoding either sGP, GP, or a plasmid control (Xu, L. et al., *Nat. Med.* (1997) in press) were transfected into 293 cells, and cell culture supernatants were used as a source of relevant recombinant glycoproteins. Binding of sGP was determined by immunofluorescence analysis after incubation of relevant supernatants with normal or transformed human cell lines. No binding was detected to several hematopoietic lineages, including lymphocytes or monocytes (Fig. 1A), or transformed Jurkat or CEM T leukemias, the HL60 myelomonocytic or U937 promonocytic leukemia cells. In contrast, sGP was able to bind to granulocytic cells, as evidenced by FACS analysis of this subset of peripheral blood mononuclear cells (PBMC) discriminated by cell size and granularity (Fig. 1A). This cell specificity was confirmed by using double-staining with a granulocyte-specific cell surface marker, CD15 (Fig. 1B). Absorption of sGP

by purified neutrophils in the absence of antibodies also resulted in depletion of sGP, indicating that binding to the neutrophil occurred in the absence of antibody (Fig. 1C).

A potential structural similarity between Ebola GP and avian sarcoma virus envelope protein has been previously proposed (Gallaher, W.R., *Cell* 85:477 (1996)), raising the possibility that this protein could be incorporated into retroviral particles. To determine the binding specificity of the transmembrane glycoprotein, pseudotyping of a Moloney leukemia virus was therefore attempted. Infectivity of different cell types by this pseudotyped vector was determined with a luciferase reporter gene. de Wet, J.R. et al., *Mol. Cell. Biol.* 7:725 (1987). This analysis revealed infection of cells different from those which interacted with sGP (Fig. 2A,B). For example, though it could infect other cell types, transduction by the GP retroviral vector readily occurred in endothelial cells, either from the microvasculature (MVEC) or umbilical veins (HUVEC) (Fig. 2B), which did not bind sGP (Fig. 2C, left). When the specificity of GP-retrovirus was compared to murine retroviruses pseudotyped with amphotropic or ecotropic envelope gp70 proteins, the range of susceptible target cells differed (Fig. 2B), suggesting that the virus receptor(s) for Ebola GP differ from those previously described for gp70. Minimal binding of GP-virus was observed on neutrophils, despite the ability of these cells to bind sGP (Fig. 2C, upper panel) and the fact that immunoreactive protein was detected on the virus. Conversely, GP-virus binding to endothelial cells was readily detected, though these cells did not bind sGP (Fig. 2C, lower panel). When sGP was analyzed for its effect on GP retroviral gene transfer, infection was not inhibited by sGP (Fig. 2D), further confirming the divergent specificities of the two forms of the viral glycoprotein. Recent studies have revealed that the biochemical forms of these proteins differ, with sGP present in solution primarily as a homodimer and GP as a trimer, suggesting that differences in multimer composition may contribute to these alternative specificities.

Potential cell surface receptors for sGP were analyzed with antibodies to several neutrophil cell surface antigens to interfere with sGP binding, including CD15, L-selectin (CD62L), CD16b, and several common leukocyte antigens. Only the neutrophil-specific form of the low affinity F_c γreceptor III, CD16b, inhibited sGP binding specifically. Antibodies to CD62L, for example, did not inhibit sGP binding (Fig. 3). Binding to neutrophils correlated with their activation state and CD16b expression since no binding was observed in cells stimulated with phorbol 12-myristate 13-acetate (PMA) for 30 minutes, at which time CD16b expression was markedly decreased on these cells (Fig. 3, lower panel). Overexpression of this form

of CD16 on a heterologous cell type, 3T3 fibroblasts, did not confer sGP binding to these cells by FACS analysis, suggesting that CD16b is necessary but not sufficient for stable binding.

Binding of sGP did not inhibit neutrophil activation in response to potent pleiotropic activators (PMA, IL-8, or f-Met-Leu-Phe), as measured by down modulation of L-selectin expression using FACS analysis. In a defined serum-free medium, partial activation of neutrophils was observed, with a decrease in L-selectin expression at 4 hours (Fig. 4). Under these conditions, incubation of neutrophils with sGP supernatant prevented this decrease in L-selectin expression (Fig. 4). Because L-selectin was not required for sGP binding (Fig. 3), this effect was apparently indirect, through a mechanism not yet defined, possibly involving CD16b or carbohydrate interactions of the highly glycosylated sGP protein.

The expression of alternative Ebola virus glycoproteins in clinical infection has long been recognized, but their functional roles and cell specificity have not been defined. Early after infection, high levels of the secreted protein are found in the serum and precede fulminant replication and dissemination of virus systemically, at which time synthesis of transmembrane GP is markedly increased. Sanchez, A. et al., *PNAS (USA)* 93:3602 (1996). The inventors have now found that the binding specificities of these two molecules differ. It had been proposed that sGP may serve as a decoy to prevent recognition of GP, possibly to temporarily inhibit virus binding to target cells. The studies set forth herein suggest that this hypothesis is unlikely to be correct. The binding specificities of these proteins differ, and despite the fact that they are derived from the same viral gene, it has been surprisingly found that alternative forms of the glycoprotein have been selected for different functions.

Although these proteins share identical amino terminal sequences, their carboxyl terminal regions differ. Sanchez, A. et al., *Virus Res.* 29:215 (1993). These sequences are likely responsible for the differences in binding specificity, either through direct interactions in these domains or by their effect on multimerization. The secreted glycoprotein binds to neutrophils to prevent early events in activation, possibly serving to diminish any inflammatory responses which might provide innate immunity to the virus, facilitating productive viral replication. The subsequent increase in GP synthesis gives rise to virus which in turn could infect other cells. Filoviruses have been shown previously to infect and replicate in different cell types and appear to grow readily in endothelial cells *in vivo*. Peters, C.J. et al., *Fields Virology*, B.N. Fields, D.M. Knipe and P.M. Howley, Eds. (Lippincott-Raven, Philadelphia) (1996);

Schnittler, H.J. et al., *J. Clin. Invest.* 91:1301 (1993). The findings set forth herein suggest that its tropism for this cell type is probably determined by the specificity of GP. In Ebola infection, preferential binding and infection of microvascular endothelial cells may lead ultimately to a loss of capillary integrity that results in the severe hemorrhage observed in the terminal stages of this disease. The differential binding of these two gene products from the same viral structural gene generated by RNA editing suggests that they have evolved functionally to differentially affect immunity and infectivity. The ability to facilitate viral replication and target the virus to endothelial cells by alternative products of the same viral gene represents an efficient genetic mechanism which can account for different pathologic features of this disease. Inhibition of sGP binding to neutrophils and GP to endothelium is likely to ameliorate the effects of acute Ebola virus infection.

SPECIFIC EXAMPLE 2

I. Methods

Production of pseudotyped MuLV retroviruses expressing green fluorescent protein (GFP): 50% - 70% confluent 293 T cells in 60mm tissue culture dishes were transfected using the calcium phosphate method and the following plasmids: 0.3 μg 1012 GP(Z) (see Figure 8) or 1012 sGp-Gp(Z) (see Figure 9), 3 μg LZR-gfp, 2 μg pNGVL-gag-pol. After overnight transfection, fresh media was added to cells. Twenty hours later, the supernatants were harvested and filtered through a .45 μm filter.

Infection of HUVEC cells using the pseudotyped retroviruses: The day before infection, 30% - 50% confluent HUVEC cells were prepared in 6-well plates. 1 ml of pseudotyped retroviral supernatant was added to one well of the 6-well plates with 15 μg/ml of polybrene. Sixteen hours later, the viruses were removed and normal media was added. After 24 hours, the cells were lifted and GFP expression measured using FACS analysis.

Construction of 1012 sGP-GP(Z): 1012 sGP(Z) cells were digested with PstI and treated with Klenow, then digested with XbaI. 1012 GP(Z) cells were digested by EcoRI and treated with Klenow, then digested with KpnI. PstI/Klenow/XbaI treated sGP fragment and EcoRI/Klenow/KpnI treated GP fragment were then cloned into XbaI/KpnI treated pVR-1012 plasmid.

GP and sGP derivatives: The receptor recognition domain, mucin-like domain and/or TM domain of GP and sGP were mutated. The mutated GP and sGP was then tested for its ability to pseudotype and for cytotoxicity in producer cells.

II. Results

To determine the efficacy of targeting endothelium with the gene transfer vectors pseudotyped with GP of the present invention, HUVEC cells were infected with GP(Z) pseudotyped MuLV retrovirus (LZR-gfp) and sGP-GP(Z) pseudotyped MuLV retrovirus (LZR-gfp). Figures 5A-5C show the infection rate (GFP expression) measured using FACS analysis. As shown in Figure 5B, the GP(Z) pseudotyped MuLV retrovirus (LZR-gfp) was effective in targeting and expressing GFP in endothelium.

To determine whether mutating GP would effect its ability to pseudotype and/or decrease toxicity in producer cells, the receptor recognition domain, mucin-like domain and/or TM domain were mutated. Figure 10 shows the results. The optimal envelope is able to pseudotype but shows minimal toxicity.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

All patents and other references cited herein are incorporated by reference as if fully set forth.

WE CLAIM:

1. A genetic construct comprising a gene operatively-linked to a carrier, wherein the carrier is associated with a transmembrane form of viral glycoprotein or derivative thereof.

2. The genetic construct of Claim 1, wherein the transmembrane form of viral glycoprotein or derivative thereof is expressed on the surface of the carrier.

3. The genetic construct of Claim 1, wherein the transmembrane form of viral glycoprotein or derivative thereof is from Ebola.

4. The genetic construct of Claim 1, wherein the carrier is a viral vector.

5. The genetic construct of Claim 1, wherein the carrier is a non-biologic gene targeting vehicle.

6. The genetic construct of Claim 4, wherein the viral vector is a retroviral vector.

7. The genetic construct of Claim 4, wherein the viral vector is a lentiviral vector.

8. The genetic construct of Claim 5, wherein the non-biologic gene targeting vehicle is a liposome.

9. The genetic construct of Claim 5, wherein the non-biologic gene targeting vehicle is a DNA-protein complex.

10. A method of targeting a gene to a cell comprising the step of administering to a cell population a genetic construct comprising the gene operatively-linked to a carrier, wherein the carrier is associated with a transmembrane form of viral glycoprotein or derivatives thereof.

11. The method of Claim 10, wherein the transmembrane form of viral glycoprotein or derivative thereof is expressed on the surface of the carrier.

12. The method of Claim 10, wherein the transmembrane form of viral glycoprotein or derivative thereof is from Ebola.

13. The method of Claim 10, wherein the carrier is a viral vector.

14. The method of Claim 10, wherein the step of administration is *ex vivo*.

15. The method of Claim 10, wherein the step of administration is *in vivo*.

16. The method of Claim 10, wherein the cell is an endothelial cell.

17. The method of Claim 10, wherein the cell is a hepatocyte.

18. The method of Claim 10, wherein the cell is a monocyte.

19. The method of Claim 10, wherein the cell is a dendritic cell.

20. The method of Claim 14, further comprising the step of introducing the cell population to a subject.

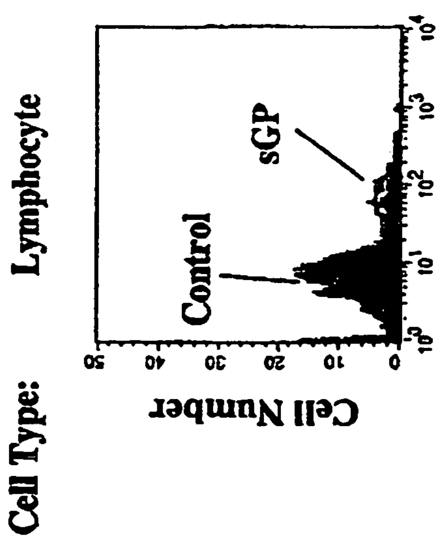


Figure 1A

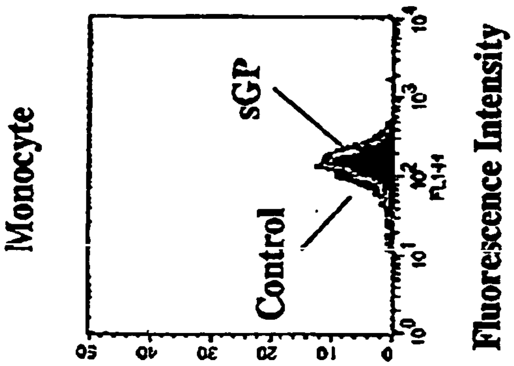


Figure 1A1

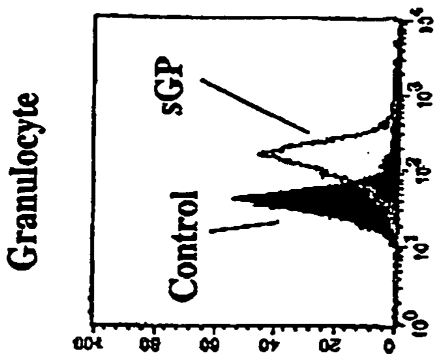


Figure 1A2

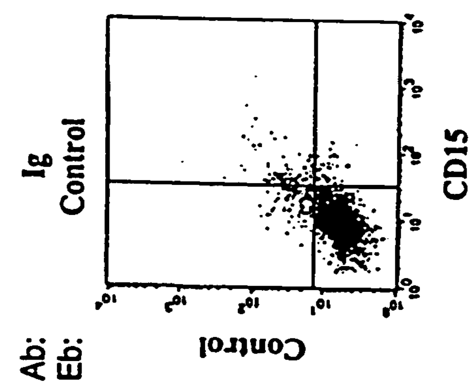


Figure 1B

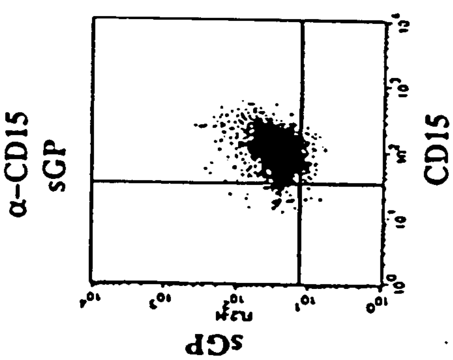


Figure 1B1

Figure 1C

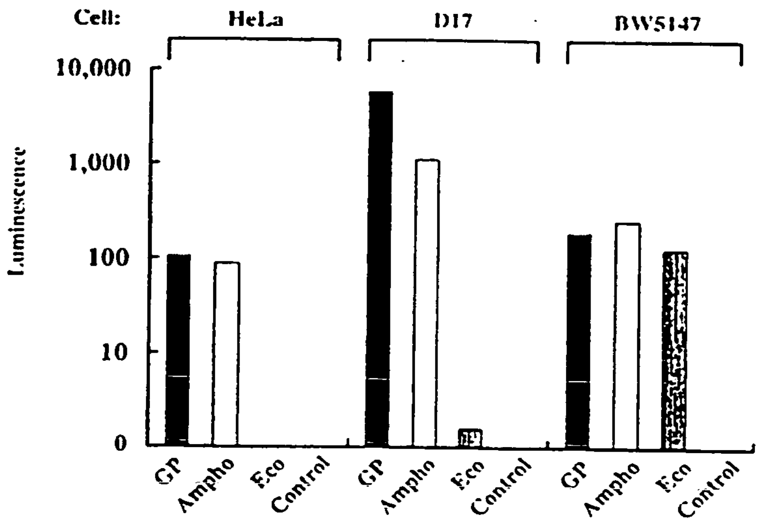


Figure 2A

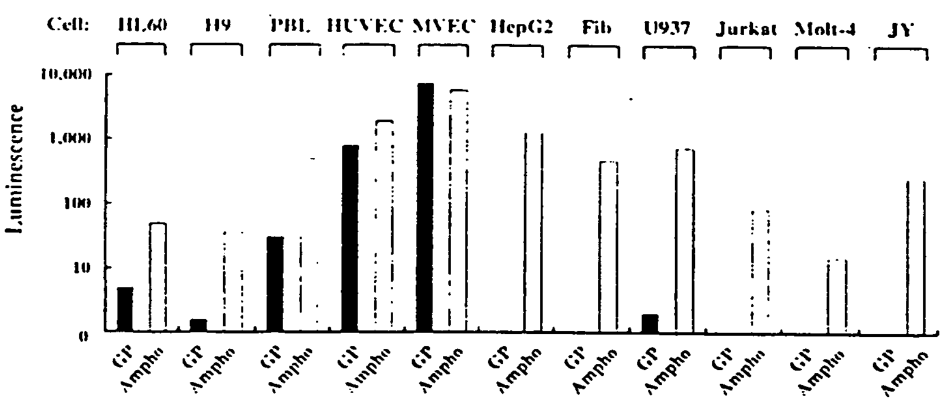


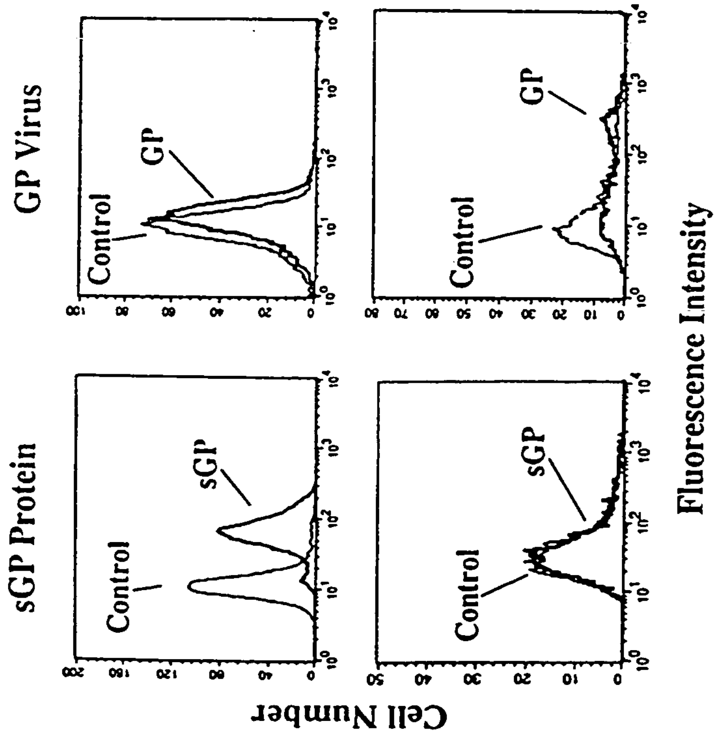
Figure 2B

Figure 2C

Figure 2C1

Figure 2C2

Figure 2C3



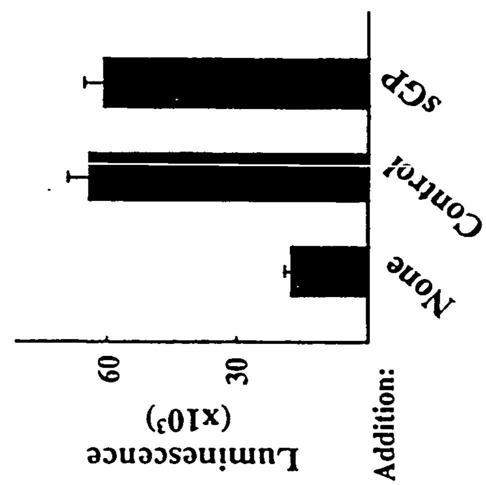


Figure 2D

Figure 3A

Ab: Control (Ig)

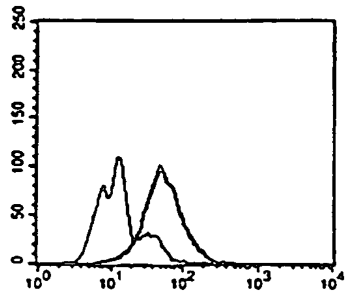


Figure 3B

α-CD16

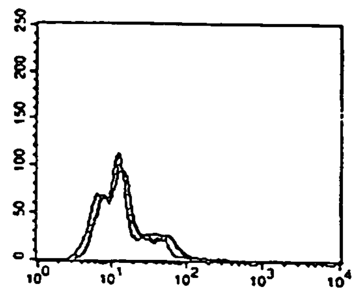


Figure 3C

Ab: Control (Ig)

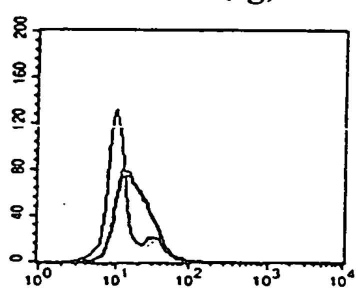
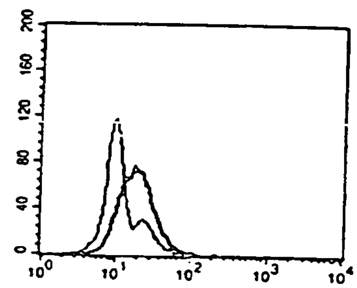


Figure 3D

α-CD62L



Cell Number

Figure 3E

Stim: None

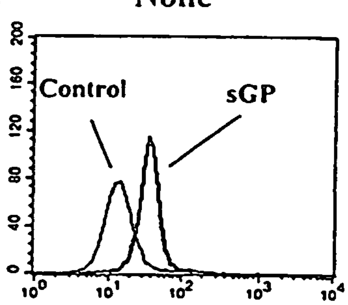
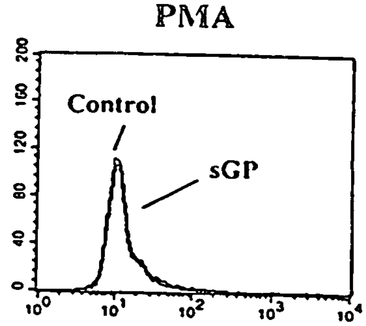


Figure 3F

PMA



Fluorescence Intensity

Figure 4A

Figure 4B

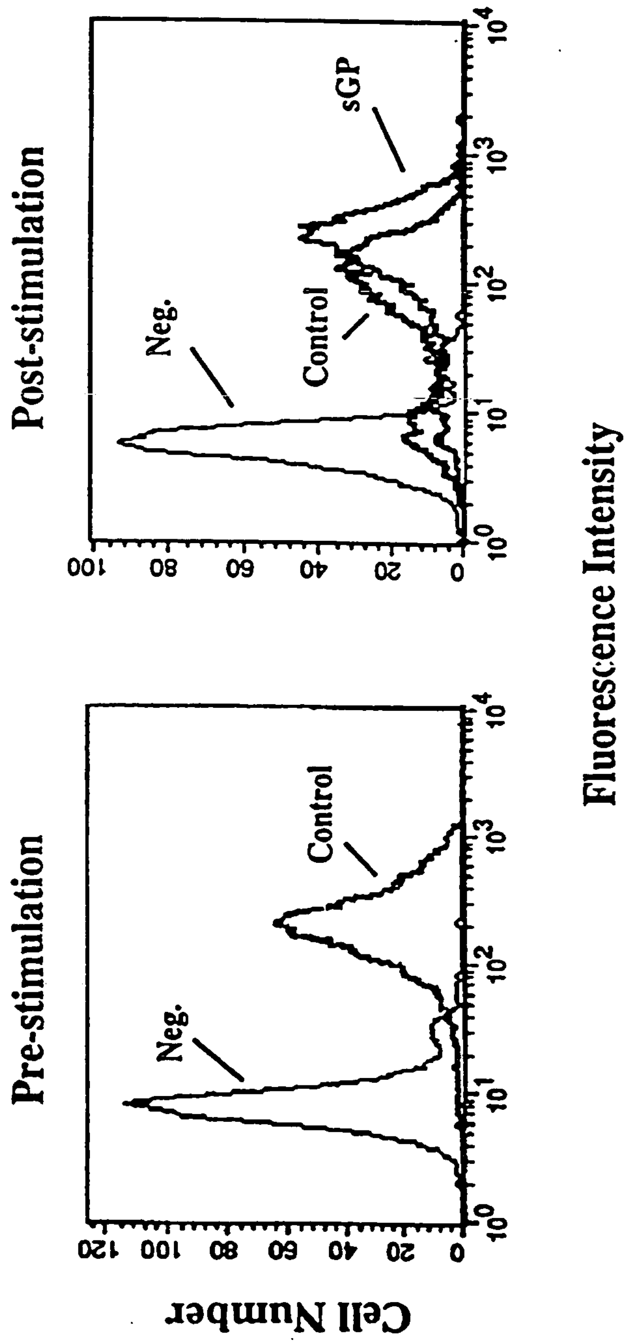
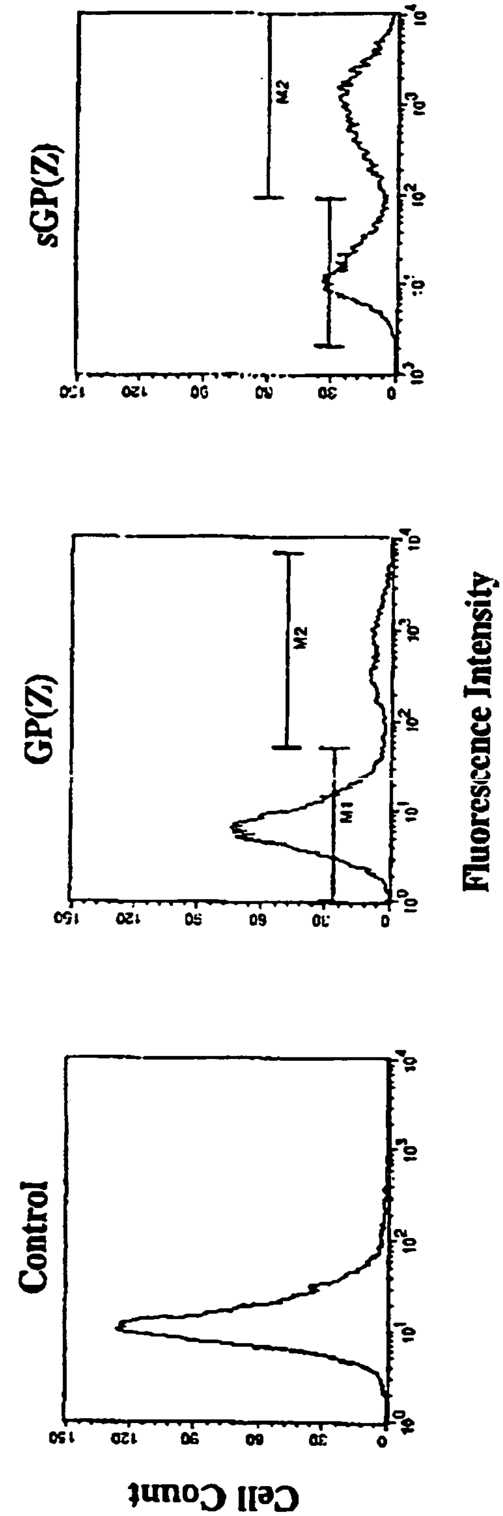


Figure 5A

Figure 5B

Figure 5C



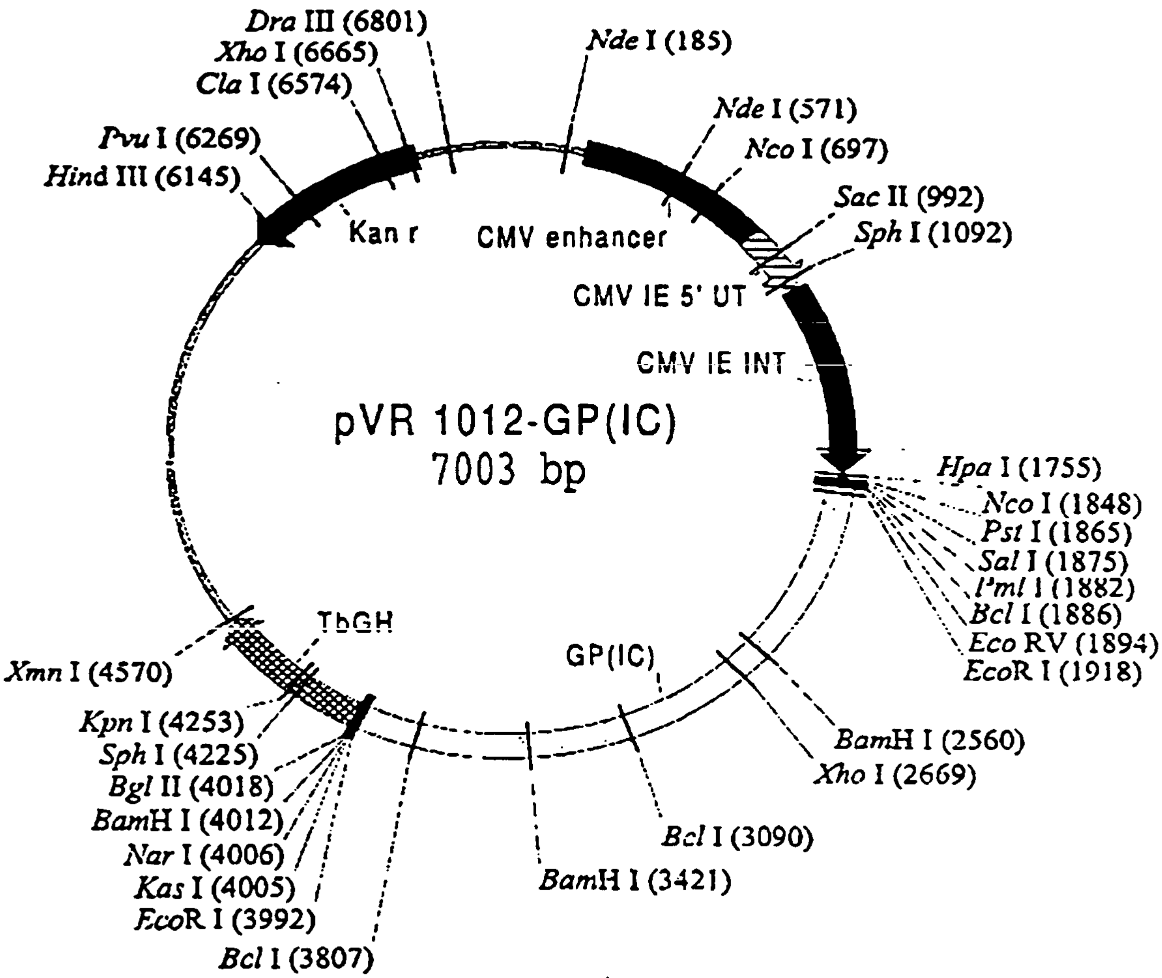
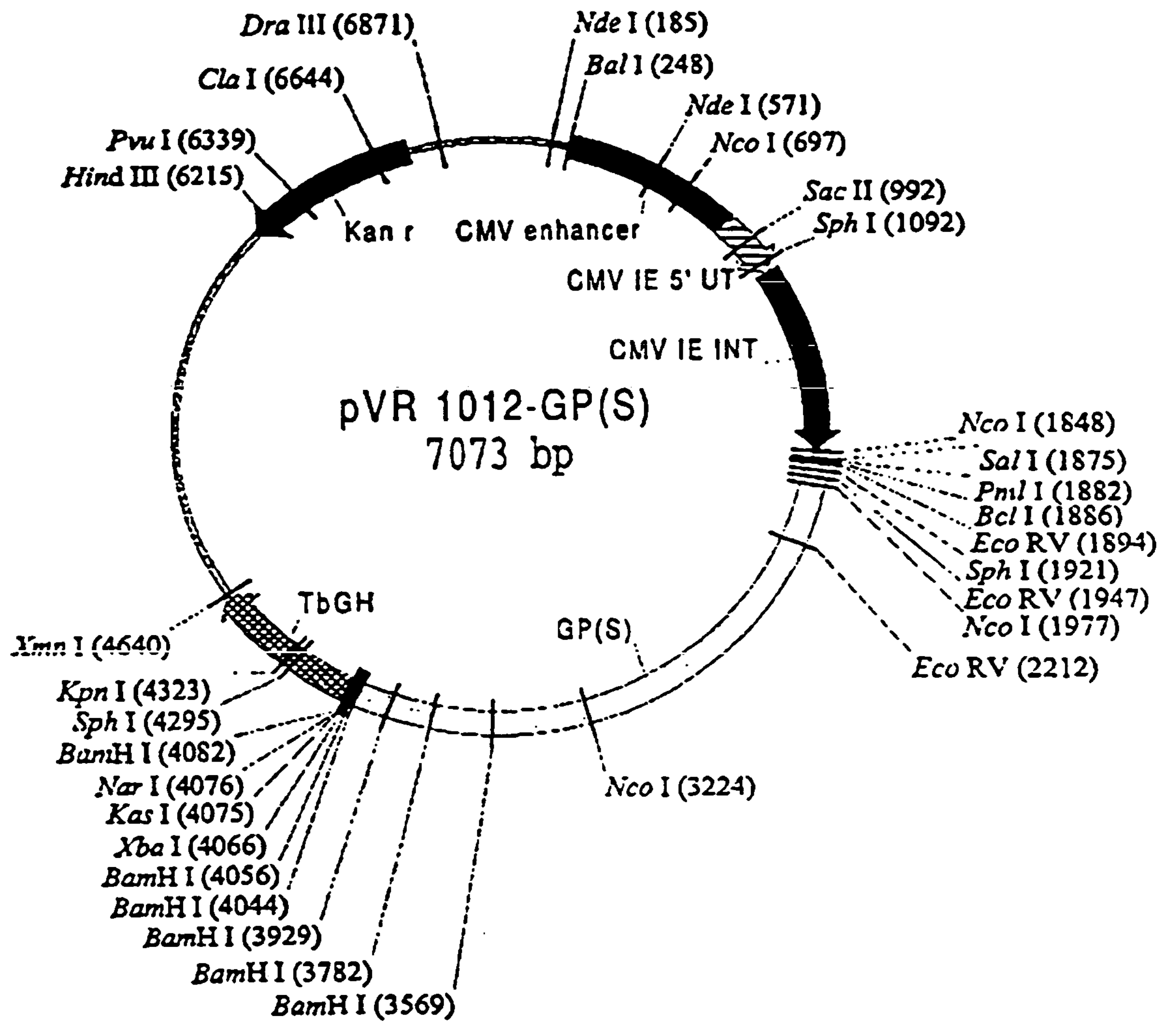

Dra III (6801)
Xho I (6665)
Cla I (6574)
Pvu I (6269)
Hind III (6145)
Nde I (185)
Nde I (571)
Nco I (697)
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-GP(IC)
7003 bp
Hpa I (1755)
Nco I (1848)
Pst I (1865)
Sal I (1875)
Pml I (1882)
Bcl I (1886)
Eco RV (1894)
EcoR I (1918)
TbGH
GP(IC)
Xmn I (4570)
Kpn I (4253)
Sph I (4225)
Bgl II (4018)
BamH I (4012)
Nar I (4006)
Kas I (4005)
EcoR I (3992)
Bcl I (3807)
BamH I (3421)
Bcl I (3090)
BamH I (2560)
Xho I (2669)

Figure 6



Dra III (6871)
Nde I (185)
Bal I (248)
Cla I (6644)
Nde I (571)
Nco I (697)
Pvu I (6339)
Hind III (6215)
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-GP(S)
7073 bp
Nco I (1848)
Sal I (1875)
Pml I (1882)
Bcl I (1886)
Eco RV (1894)
Sph I (1921)
Eco RV (1947)
Nco I (1977)
Eco RV (2212)
TbGH
GP(S)
Xmn I (4640)
Kpn I (4323)
Sph I (4295)
BamH I (4082)
Nar I (4076)
Kas I (4075)
Xba I (4066)
BamH I (4056)
BamH I (4044)
BamH I (3929)
BamH I (3782)
BamH I (3569)
Nco I (3224)

Figure 7

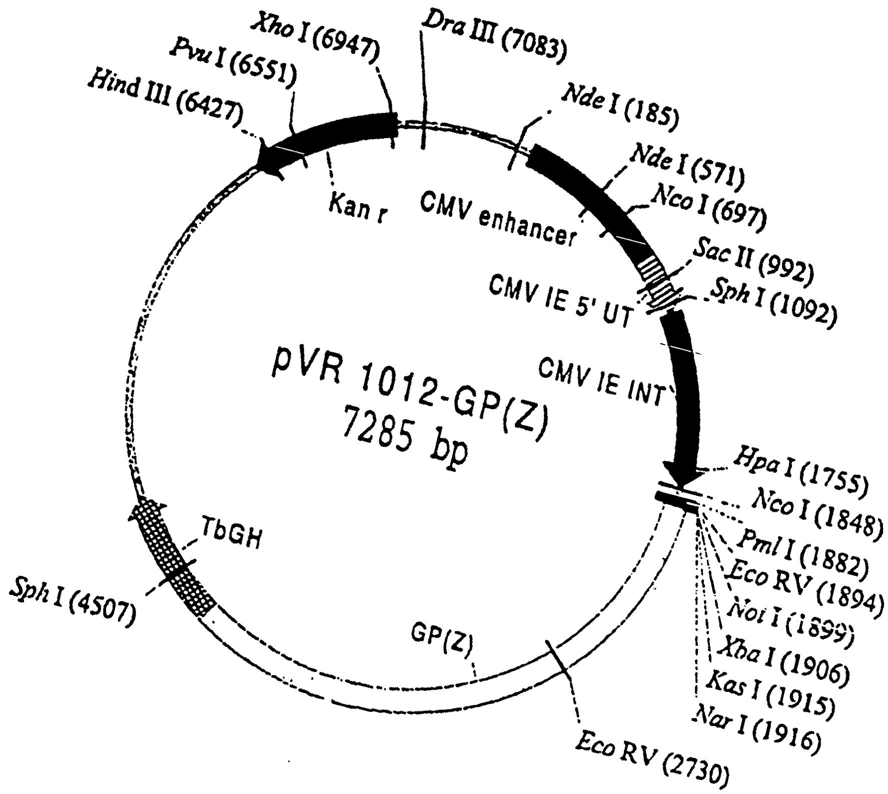

Xho I (6947)
Dra III (7083)
Pvu I (6551)
Hind III (6427)
Nde I (185)
Nde I (571)
Nco I (697)
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-GP(Z)
7285 bp
Hpa I (1755)
Nco I (1848)
Pml I (1882)
Eco RV (1894)
Not I (1899)
Xba I (1906)
Kas I (1915)
Nar I (1916)
TbGH
Sph I (4507)
GP(Z)
Eco RV (2730)

Figure 8

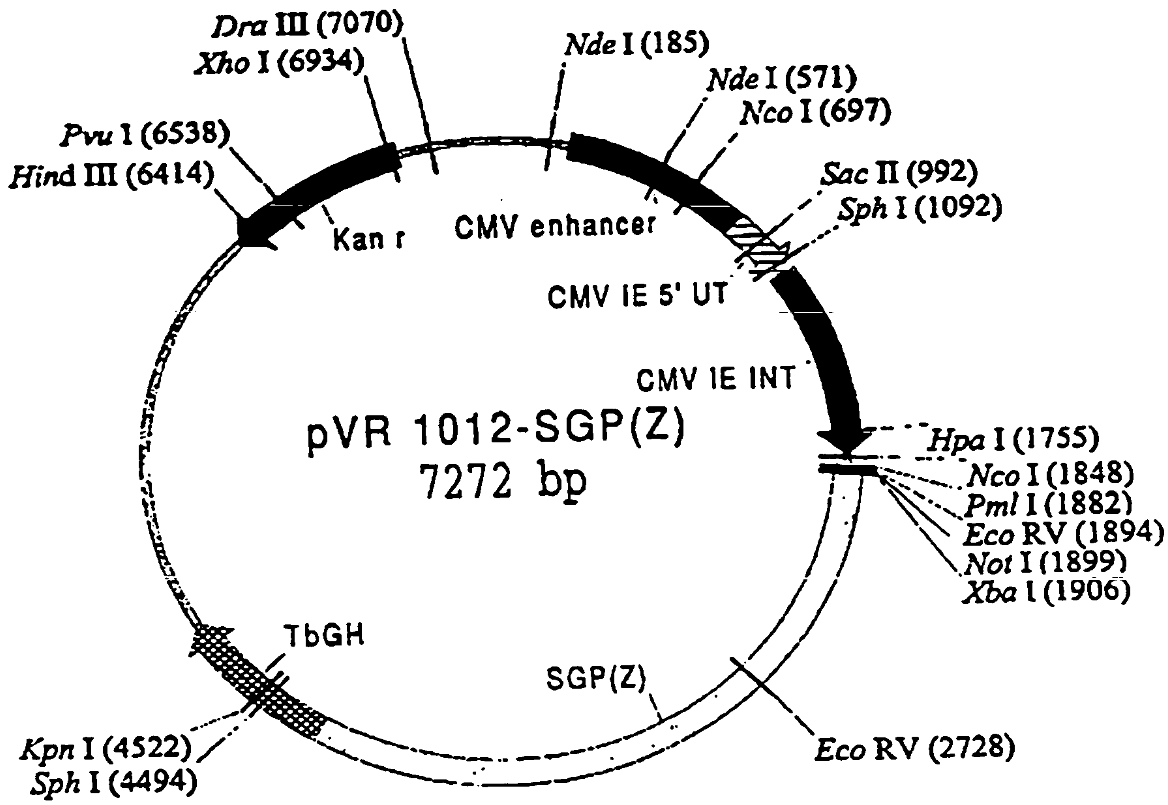

Dra III (7070)
Xho I (6934)
Nde I (185)
Nde I (571)
Nco I (697)
Pvu I (6538)
Hind III (6414)
Sac II (992)
Sph I (1092)
Kan r
CMV enhancer
CMV IE 5' UT
CMV IE INT
pVR 1012-SGP(Z)
7272 bp
Hpa I (1755)
Nco I (1848)
Pml I (1882)
Eco RV (1894)
Not I (1899)
Xba I (1906)
TbGH
SGP(Z)
Kpn I (4522)
Sph I (4494)
Eco RV (2728)

Figure 9

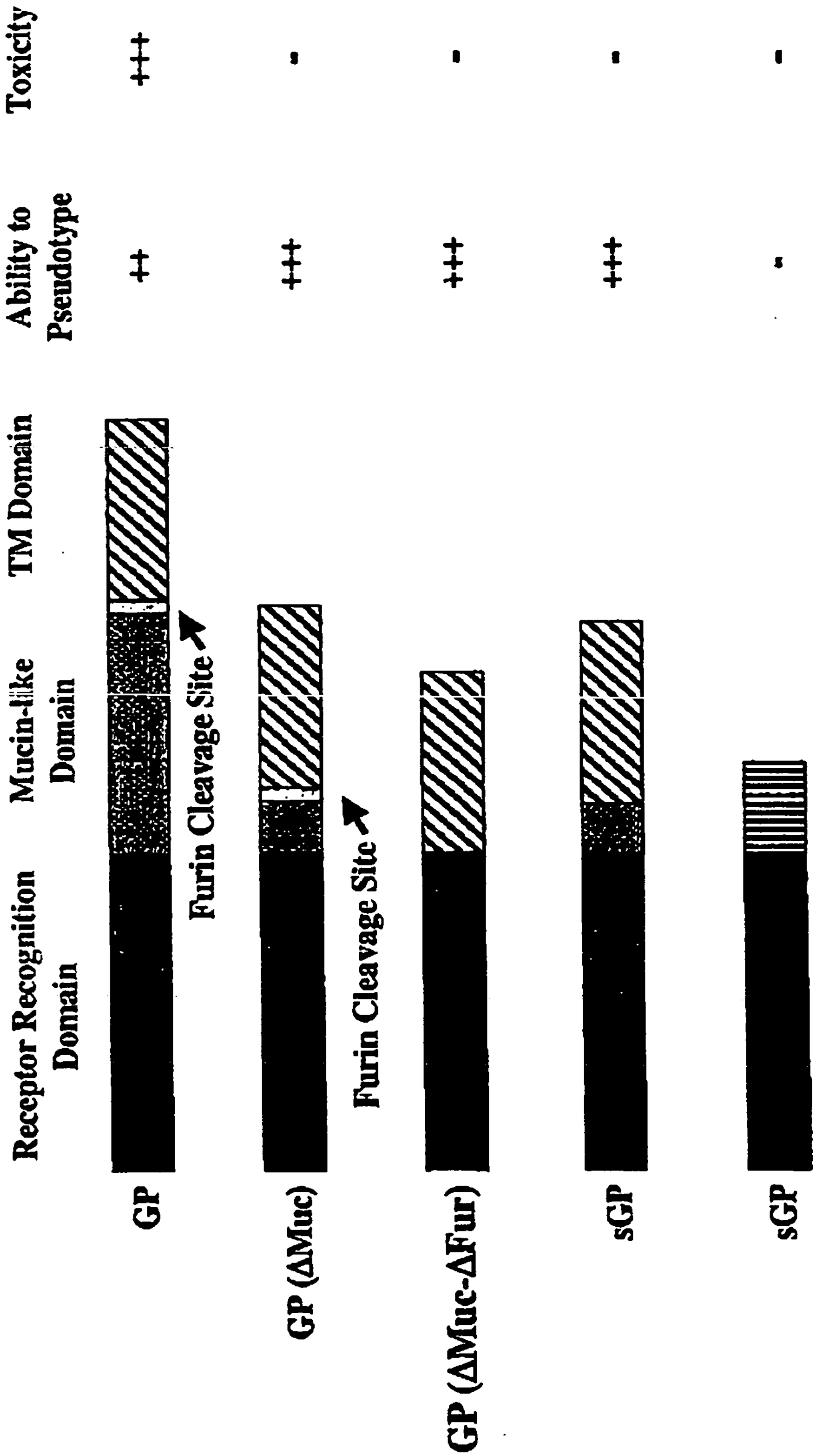

Receptor Recognition Domain
Mucin-like Domain
TM Domain
Ability to Pseudotype
Toxicity
GP
++
+++
Furin Cleavage Site
GP (ΔMuc)
+++
-
Furin Cleavage Site
GP (ΔMuc-ΔFur)
+++
-
sGP
+++
-
sGP
-
-

Figure 10

SEQUENCE LISTING ID NO: 1

pVR 1012-GP(IC)

General Description

DNA pVR 1012-GP(IC)
Local object
Created: 09/14/98 04:17PM
Last Modification Date: ? (no data)
length: 7003 bp
storage type: Basic
form: Circular

Comments

Restriction Map

BglII: 1 site AGATCT
TCTAGA

ClaI: 1 site ATCGAT
TAGCTA

DraIII: 1 site CACNNNGTG
GTGNNNCAC

EcoRV: 1 site GATATC
CTATAG

HindIII: 1 site AAGCTT
TTCGAA

HpaI: 1 site GTTAAC
CAATTG

KasI: 1 site GGCGCC
CCGCGG

KpnI: 1 site GGTACC
CCATGG

NarI: 1 site GGCGCC
CCGCGG

PmlI: 1 site CACGTG
GTGCAC

PstI: 1 site CTGCAG
GACGTC

PvuI: 1 site CGATCG
GCTAGC

SacII: 1 site CCGCGG
GGCGCC

SalI: 1 site GTCGAC
CAGCTG

XmnI: 1 site GAANNNNTTC
CTTNNNNAAG

EcoRI: 2 sites GAATTC
CTTAAG

NcoI: 2 sites CCATGG
GGTACC

NdeI: 2 sites CATATG
GTATAC

SphI: 2 sites GCATGC
CGTACG

XhoI: 2 sites CTCGAG
GAGCTC

BamHI: 3 sites GGATCC
CCTAGG

BclI: 3 sites TGATCA
ACTAGT

Functional Map

CDS (4 signals)

CMV IE 5' UT

Start: 886 End: 1129

CMV IE INT

Start: 1130 End: 1840

TbGH

Start: 4020 End: 4572

Kan r

Start: 6068 End: 6690 (Complementary)

Misc_feature (2 signals)

CMV enhancer

Start: 248 End: 885

GP(IC)

Start: 1870 End: 4019

Annotations

```
1    TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

51   GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                      NdeI
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                      NdeI
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                    NcoI
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
```

851 TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC

901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA

SacII

951 TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT

1001 CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG

SphI

1051 CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA

1101 TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC

1151 TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG

1201 TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT

1251 CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG

1301 ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA

1351 CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT

1401 GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA

1451 TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG

1501 AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC

1551 ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC

1601 TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC

1651 GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC

1701 CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG

```
      HpaI
1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

                                                    NcoI
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

                                 SalI
      NcoI      PstI                    PmlI   BclI    EcoRV
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

                      EcoRI
1901  CCGCGCGGCC GCTCTAGAAT TCTCTAATCA CAGTCATCAT GGGAGCGTCA
      GGCGCGCCGG CGAGATCTTA AGAGATTAGT GTCAGTAGTA CCCTCGCAGT

1951  GGGATTCTGC AATTGCCCCG TGAGCGCTTC AGGAAAACAT CTTTCTTTGT
      CCCTAAGACG TTAACGGGGC ACTCGCGAAG TCCTTTTGTA GAAAGAAACA

2001  TTGGGTAATA ATCCTATTCC ATAAAGTCTT TTCAATCCCG TTGGGGGTTG
      AACCCATTAT TAGGATAAGG TATTTCAGAA AAGTTAGGGC AACCCCCAAC

2051  TACACAACAA TACCCTACAA GTGAGTGATA TTGACAAGTT TGTGTGCCGA
      ATGTGTTGTT ATGGGATGTT CACTCACTAT AACTGTTCAA ACACACGGCT

2101  GACAAACTCT CTTCAACTAG CCAATTGAAG TCAGTCGGGT TGAACTTGGA
      CTGTTTGAGA GAAGTTGATC GGTTAACTTC AGTCAGCCCA ACTTGAACCT

2151  GGGCAATGGA GTAGCAACTG ATGTACCAAC GGCAACCAAA AGATGGGGTT
      CCCGTTACCT CATCGTTGAC TACATGGTTG CCGTTGGTTT TCTACCCCAA

2201  TTCGAGCTGG TGTTCCACCA AAGGTGGTAA ATTACGAAGC TGGAGAATGG
      AAGCTCGACC ACAAGGTGGT TTCCACCATT TAATGCTTCG ACCTCTTACC

2251  GCTGAGAACT GTTATAACCT GGCTATAAAG AAAGTTGATG GTAGTGAGTG
      CGACTCTTGA CAATATTGGA CCGATATTTC TTTCAACTAC CATCACTCAC

2301  CCTACCAGAA GCCCCTGAGG GAGTGAGGGA TTTTCCCCGT TGCCGCTATG
      GGATGGTCTT CGGGGACTCC CTCACTCCCT AAAAGGGGCA ACGGCGATAC

2351  TACACAAAGT CTCAGGAACT GGACCATGCC CAGGAGGACT CGCCTTTCAC
      ATGTGTTTCA GAGTCCTTGA CCTGGTACGG GTCCTCCTGA GCGGAAAGTG

2401  AAAGAAGGAG CCTTCTTCCT GTATGACCGA CTCGCATCAA CAATCATTTA
      TTTCTTCCTC GGAAGAAGGA CATACTGGCT GAGCGTAGTT GTTAGTAAAT

2451  TCGGGGTACA ACCTTTGCCG AAGGAGTTAT TGCATTTCTG ATCTTGCCTA
      AGCCCCATGT TGGAAACGGC TTCCTCAATA ACGTAAAGAC TAGAACGGAT

2501  AGGCGCGAAA GGATTTTTTC CAGTCTCCTC CATTGCATGA GCCTGCCAAC
      TCCGCGCTTT CCTAAAAAAG GTCAGAGGAG GTAACGTACT CGGACGGTTG
```

```
              BamHI
2551  ATGACCACGG ATCCCTCCAG TTACTATCAC ACGACAACAA TAAACTACGT
      TACTGGTGCC TAGGGAGGTC AATGATAGTG TGCTGTTGTT ATTTGATGCA

2601  GGTTGATAAT TTTGGAACCA ACACCACAGA GTTTCTGTTC CAAGTCGATC
      CCAACTATTA AAACCTTGGT TGTGGTGTCT CAAAGACAAG GTTCAGCTAG

                    XhoI
2651  ATTTGACGTA TGTGCAGCTC GAGGCAAGAT TCACACCACA ATTCCTTGTC
      TAAACTGCAT ACACGTCGAG CTCCGTTCTA AGTGTGGTGT TAAGGAACAG

2701  CTCCTAAATG AAACCATCTA CTCTGATAAC CGCAGAAGTA ACACAACAGG
      GAGGATTTAC TTTGGTAGAT GAGACTATTG GCGTCTTCAT TGTGTTGTCC

2751  AAAACTAATC TGGAAAATAA ATCCCACTGT TGATACCAGC ATGGGTGAGT
      TTTTGATTAG ACCTTTTATT TAGGGTGACA ACTATGGTCG TACCCACTCA

2801  GGGCTTTCTG GGAAAATAAA AAAACTTCAC AAAAACCCTT TCAAGTGAAG
      CCCGAAAGAC CCTTTTATTT TTTTGAAGTG TTTTTGGGAA AGTTCACTTC

2851  AGTTGTCTTT CGTACCTGTA CCAGAAACCC AGAACCAGGT CCTTGACACG
      TCAACAGAAA GCATGGACAT GGTCTTTGGG TCTTGGTCCA GGAACTGTGC

2901  ACAGCGACGG TCTCTCCTCC CATCTCCGCC CACAACCACG CAGGCGAAGA
      TGTCGCTGCC AGAGAGGAGG GTAGAGGCGG GTGTTGGTGC GTCCGCTTCT

2951  CCACAAAGAA TTGGTTTCAG AGGATTCCAC TCCAGTGGTT CAGATGCAAA
      GGTGTTTCTT AACCAAAGTC TCCTAAGGTG AGGTCACCAA GTCTACGTTT

3001  ACATCAAGGG AAAGGACACA ATGCCAACCA CAGTGACGGG TGTACCAACA
      TGTAGTTCCC TTTCCTGTGT TACGGTTGGT GTCACTGCCC ACATGGTTGT

                                                 BclI
3051  ACCACACCCT CTCCATTTCC AATCAATGCT CGCAACACTG ATCATACCAA
      TGGTGTGGGA GAGGTAAAGG TTAGTTACGA GCGTTGTGAC TAGTATGGTT

3101  ATCATTTATC GGCCTGGAGG GGCCCCAAGA AGACCACAGC ACCACACAGC
      TAGTAAATAG CCGGACCTCC CCGGGGTTCT TCTGGTGTCG TGGTGTGTCG

3151  CTGCCAAGAC CACCAGCCAA CCAACCAACA GCACAGAATC GACGACACTA
      GACGGTTCTG GTGGTCGGTT GGTTGGTTGT CGTGTCTTAG CTGCTGTGAT

3201  AACCCAACAT CAGAGCCCTC CAGTAGAGGC ACGGGACCAT CCAGCCCCAC
      TTGGGTTGTA GTCTCGGGAG GTCATCTCCG TGCCCTGGTA GGTCGGGGTG

3251  GGTCCCCAAC ACCACAGAAA GCCACGCCGA ACTTGGCAAG ACAACCCCAA
      CCAGGGGTTG TGGTGTCTTT CGGTGCGGCT TGAACCGTTC TGTTGGGGTT

3301  CCACACTCCC AGAACAGCAC ACTGCCGCCA GTGCCATTCC AAGAGCCGTG
      GGTGTGAGGG TCTTGTCGTG TGACGGCGGT CACGGTAAGG TTCTCGGCAC

3351  CACCCCGACG AACTCAGTGG ACCTGGCTTC CTGACGAACA CAATACGGGG
      GTGGGGCTGC TTGAGTCACC TGGACCGAAG GACTGCTTGT GTTATGCCCC
```

```
                                 BamHI
3401  GGTGACAAAT CTCCTGACAG GATCCAGAAG AAAGCGAAGG GATGTCACTC
      CCACTGTTTA GAGGACTGTC CTAGGTCTTC TTTCGCTTCC CTACAGTGAG

3451  CCAATACACA ACCCAAATGC AACCCAAACC TGCACTATTG GACAGCCTTG
      GGTTATGTGT TGGGTTTACG TTGGGTTTGG ACGTGATAAC CTGTCGGAAC

3501  GATGAGGGTG CTGCCATAGG TTTAGCCTGG ATACCATACT TCGGGCCAGC
      CTACTCCCAC GACGGTATCC AAATCGGACC TATGGTATGA AGCCCGGTCG

3551  AGCTGAGGGA ATTTACACTG AAGGCATAAT GGAGAATCAA AATGGATTGA
      TCGACTCCCT TAAATGTGAC TTCCGTATTA CCTCTTAGTT TTACCTAACT

3601  TCTGTGGATT GAGGCAGCTG GCCAACGAAA CGACACAAGC TCTTCAATTG
      AGACACCTAA CTCCGTCGAC CGGTTGCTTT GCTGTGTTCG AGAAGTTAAC

3651  TTCTTAAGGG CAACTACTGA GTTGCGTACA TTCTCTATAC TAAATCGGAA
      AAGAATTCCC GTTGATGACT CAACGCATGT AAGAGATATG ATTTAGCCTT

3701  AGCAATAGAC TTCTTGCTCC AAAGATGGGG AGGAACATGT CACATTCTAG
      TCGTTATCTG AAGAACGAGG TTTCTACCCC TCCTTGTACA GTGTAAGATC

3751  GGCCTGATTG TTGCATTGAA CCCCAAGATT GGACCAAAAA TATCACTGAT
      CCGGACTAAC AACGTAACTT GGGGTTCTAA CCTGGTTTTT ATAGTGACTA

         BclI
3801  AAAATTGATC AAATAATCCA TGACTTTGTC GATAATAATC TTCCAAATCA
      TTTTAACTAG TTTATTAGGT ACTGAAACAG CTATTATTAG AAGGTTTAGT

3851  GAATGATGGC AGCAACTGGT GGACTGGATG GAAACAATGG GTTCCTGCTG
      CTTACTACCG TCGTTGACCA CCTGACCTAC CTTTGTTACC CAAGGACGAC

3901  GAATAGGAAT CACAGGAGTA ATCATTGCTA TTATTGCTTT GCTGTGCATT
      CTTATCCTTA GTGTCCTCAT TAGTAACGAT AATAACGAAA CGACACGTAA

                                                 EcoRI
3951  TGCAAATTCA TGCTTTGAAC TAATATAGCA TCATACTTTA GAATTCTAGA
      ACGTTTAAGT ACGAAACTTG ATTATATCGT AGTATGAAAT CTTAAGATCT

        NarI
        KasI  BamHI BglII
4001  CCAGGCGCCT GGATCCAGAT CTGCTGTGCC TTCTAGTTGC CAGCCATCTG
      GGTCCGCGGA CCTAGGTCTA GACGACACGG AAGATCAACG GTCGGTAGAC

4051  TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC
      AACAAACGGG GAGGGGGCAC GGAAGGAACT GGGACCTTCC ACGGTGAGGG

4101  ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG
      TGACAGGAAA GGATTATTTT ACTCCTTTAA CGTAGCGTAA CAGACTCATC

4151  GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGCACAGC AAGGGGGAGG
      CACAGTAAGA TAAGACCCCC CACCCCACCC CGTCGTGTCG TTCCCCCTCC
```

```
                         SphI                 KpnI
4201  ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGGT
      TAACCCTTCT GTTATCGTCC GTACGACCCC TACGCCACCC GAGATACCCA

      KpnI
4251  ACCCAGGTGC TGAAGAATTG ACCCGGTTCC TCCTGGGCCA GAAAGAAGCA
      TGGGTCCACG ACTTCTTAAC TGGGCCAAGG AGGACCCGGT CTTTCTTCGT

4301  GGCACATCCC CTTCTCTGTG ACACACCCTG TCCACGCCCC TGGTTCTTAG
      CCGTGTAGGG GAAGAGACAC TGTGTGGGAC AGGTGCGGGG ACCAAGAATC

4351  TTCCAGCCCC ACTCATAGGA CACTCATAGC TCAGGAGGGC TCCGCCTTCA
      AAGGTCGGGG TGAGTATCCT GTGAGTATCG AGTCCTCCCG AGGCGGAAGT

4401  ATCCCACCCG CTAAAGTACT TGGAGCGGTC TCTCCCTCCC TCATCAGCCC
      TAGGGTGGGC GATTTCATGA ACCTCGCCAG AGAGGGAGGG AGTAGTCGGG

4451  ACCAAACCAA ACCTAGCCTC CAAGAGTGGG AAGAAATTAA AGCAAGATAG
      TGGTTTGGTT TGGATCGGAG GTTCTCACCC TTCTTTAATT TCGTTCTATC

4501  GCTATTAAGT GCAGAGGGAG AGAAAATGCC TCCAACATGT GAGGAAGTAA
      CGATAATTCA CGTCTCCCTC TCTTTTACGG AGGTTGTACA CTCCTTCATT

                     XmnI
4551  TGAGAGAAAT CATAGAATTT CTTCCGCTTC CTCGCTCACT GACTCGCTGC
      ACTCTCTTTA GTATCTTAAA GAAGGCGAAG GAGCGAGTGA CTGAGCGACG

4601  GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA
      CGAGCCAGCA AGCCGACGCC GCTCGCCATA GTCGAGTGAG TTTCCGCCAT

4651  ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC
      TATGCCAATA GGTGTCTTAG TCCCCTATTG CGTCCTTTCT TGTACACTCG

4701  AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
      TTTTCCGGTC GTTTTCCGGT CCTTGGCATT TTTCCGGCGC AACGACCGCA

4751  TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA
      AAAAGGTATC CGAGGCGGGG GGACTGCTCG TAGTGTTTTT AGCTGCGAGT

4801  AGTCAGAGGT GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC
      TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT ATTTCTATGG TCCGCAAAGG

4851  CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG
      GGGACCTTCG AGGGAGCACG CGAGAGGACA AGGCTGGGAC GGCGAATGGC

4901  GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC
      CTATGGACAG GCGGAAAGAG GGAAGCCCTT CGCACCGCGA AAGAGTTACG

4951  TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG
      AGTGCGACAT CCATAGAGTC AAGCCACATC CAGCAAGCGA GGTTCGACCC

5001  CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
      GACACACGTG CTTGGGGGGC AAGTCGGGCT GGCGACGCGG AATAGGCCAT
```

```
5051 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA
     TGATAGCAGA ACTCAGGTTG GGCCATTCTG TGCTGAATAG CGGTGACCGT

5101 GCAGCCACTG GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC
     CGTCGGTGAC CATTGTCCTA ATCGTCTCGC TCCATACATC CGCCACGATG

5151 AGAGTTCTTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT
     TCTCAAGAAC TTCACCACCG GATTGATGCC GATGTGATCT TCCTGTCATA

5201 TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT
     AACCATAGAC GCGAGACGAC TTCGGTCAAT GGAAGCCTTT TTCTCAACCA

5251 AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT
     TCGAGAACTA GGCCGTTTGT TTGGTGGCGA CCATCGCCAC CAAAAAAACA

5301 TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
     AACGTTCGTC GTCTAATGCG CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA

5351 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA
     ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA CCTTGCTTTT GAGTGCAATT

5401 GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT
     CCCTAAAACC AGTACTCTAA TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA

5451 AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT
     TTTAATTTTT ACTTCAAAAT TTAGTTAGAT TTCATATATA CTCATTTGAA

5501 GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC
     CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA GAGTCGCTAG

5551 TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCGGGGGG GGGGGGCGCT
     ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGCCCCCC CCCCCCGCGA

5601 GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG ACTCATACCA GGCCTGAATC
     CTCCAGACGG AGCACTTCTT CCACAACGAC TGAGTATGGT CCGGACTTAG

5651 GCCCCATCAT CCAGCCAGAA AGTGAGGGAG CCACGGTTGA TGAGAGCTTT
     CGGGGTAGTA GGTCGGTCTT TCACTCCCTC GGTGCCAACT ACTCTCGAAA

5701 GTTGTAGGTG GACCAGTTGG TGATTTTGAA CTTTTGCTTT GCCACGGAAC
     CAACATCCAC CTGGTCAACC ACTAAAACTT GAAAACGAAA CGGTGCCTTG

5751 GGTCTGCGTT GTCGGGAAGA TGCGTGATCT GATCCTTCAA CTCAGCAAAA
     CCAGACGCAA CAGCCCTTCT ACGCACTAGA CTAGGAAGTT GAGTCGTTTT

5801 GTTCGATTTA TTCAACAAAG CCGCCGTCCC GTCAAGTCAG CGTAATGCTC
     CAAGCTAAAT AAGTTGTTTC GGCGGCAGGG CAGTTCAGTC GCATTACGAG

5851 TGCCAGTGTT ACAACCAATT AACCAATTCT GATTAGAAAA ACTCATCGAG
     ACGGTCACAA TGTTGGTTAA TTGGTTAAGA CTAATCTTTT TGAGTAGCTC

5901 CATCAAATGA AACTGCAATT TATTCATATC AGGATTATCA ATACCATATT
     GTAGTTTACT TTGACGTTAA ATAAGTATAG TCCTAATAGT TATGGTATAA

5951 TTTGAAAAAG CCGTTTCTGT AATGAAGGAG AAAACTCACC GAGGCAGTTC
     AAACTTTTTC GGCAAAGACA TTACTTCCTC TTTTGAGTGG CTCCGTCAAG
```

```
6001  CATAGGATGG CAAGATCCTG GTATCGGTCT GCGATTCCGA CTCGTCCAAC
      GTATCCTACC GTTCTAGGAC CATAGCCAGA CGCTAAGGCT GAGCAGGTTG

6051  ATCAATACAA CCTATTAATT TCCCCTCGTC AAAAATAAGG TTATCAAGTG
      TAGTTATGTT GGATAATTAA AGGGGAGCAG TTTTTATTCC AATAGTTCAC

                                                  HindIII
6101  AGAAATCACC ATGAGTGACG ACTGAATCCG GTGAGAATGG CAAAAGCTTA
      TCTTTAGTGG TACTCACTGC TGACTTAGGC CACTCTTACC GTTTTCGAAT

6151  TGCATTTCTT TCCAGACTTG TTCAACAGGC CAGCCATTAC GCTCGTCATC
      ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG GTCGGTAATG CGAGCAGTAG

6201  AAAATCACTC GCATCAACCA AACCGTTATT CATTCGTGAT TGCGCCTGAG
      TTTTAGTGAG CGTAGTTGGT TTGGCAATAA GTAAGCACTA ACGCGGACTC

                 PvuI
6251  CGAGACGAAA TACGCGATCG CTGTTAAAAG GACAATTACA AACAGGAATC
      GCTCTGCTTT ATGCGCTAGC GACAATTTTC CTGTTAATGT TTGTCCTTAG

6301  GAATGCAACC GGCGCAGGAA CACTGCCAGC GCATCAACAA TATTTTCACC
      CTTACGTTGG CCGCGTCCTT GTGACGGTCG CGTAGTTGTT ATAAAAGTGG

6351  TGAATCAGGA TATTCTTCTA ATACCTGGAA TGCTGTTTTC CCGGGGATCG
      ACTTAGTCCT ATAAGAAGAT TATGGACCTT ACGACAAAAG GGCCCCTAGC

6401  CAGTGGTGAG TAACCATGCA TCATCAGGAG TACGGATAAA ATGCTTGATG
      GTCACCACTC ATTGGTACGT AGTAGTCCTC ATGCCTATTT TACGAACTAC

6451  GTCGGAAGAG GCATAAATTC CGTCAGCCAG TTTAGTCTGA CCATCTCATC
      CAGCCTTCTC CGTATTTAAG GCAGTCGGTC AAATCAGACT GGTAGAGTAG

6501  TGTAACATCA TTGGCAACGC TACCTTTGCC ATGTTTCAGA AACAACTCTG
      ACATTGTAGT AACCGTTGCG ATGGAAACGG TACAAAGTCT TTGTTGAGAC

                            ClaI
6551  GCGCATCGGG CTTCCCATAC AATCGATAGA TTGTCGCACC TGATTGCCCG
      CGCGTAGCCC GAAGGGTATG TTAGCTATCT AACAGCGTGG ACTAACGGGC

6601  ACATTATCGC GAGCCCATTT ATACCCATAT AAATCAGCAT CCATGTTGGA
      TGTAATAGCG CTCGGGTAAA TATGGGTATA TTTAGTCGTA GGTACAACCT

                 XhoI
6651  ATTTAATCGC GGCCTCGAGC AAGACGTTTC CCGTTGAATA TGGCTCATAA
      TAAATTAGCG CCGGAGCTCG TTCTGCAAAG GGCAACTTAT ACCGAGTATT

6701  CACCCCTTGT ATTACTGTTT ATGTAAGCAG ACAGTTTTAT TGTTCATGAT
      GTGGGGAACA TAATGACAAA TACATTCGTC TGTCAAAATA ACAAGTACTA

                                                   DraIII
6751  GATATATTTT TATCTTGTGC AATGTAACAT CAGAGATTTT GAGACACAAC
      CTATATAAAA ATAGAACACG TTACATTGTA GTCTCTAAAA CTCTGTGTTG
```

```
     DraIII
     ------
6801 GTGGCTTTCC CCCCCCCCCC ATTATTGAAG CATTTATCAG GGTTATTGTC
     CACCGAAAGG GGGGGGGGGG TAATAACTTC GTAAATAGTC CCAATAACAG
.....................................................................
6851 TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG
     AGTACTCGCC TATGTATAAA CTTACATAAA TCTTTTTATT TGTTTATCCC
.....................................................................
6901 GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT
     CAAGGCGCGT GTAAAGGGGC TTTTCACGGT GGACTGCAGA TTCTTTGGTA
.....................................................................
6951 TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCACG AGGCCCTTTC
     ATAATAGTAC TGTAATTGGA TATTTTTATC CGCATAGTGC TCCGGGAAAG
.....................................................................
7001 GTC
     CAG
.....................................................................
```

pVR 1012-GP(S)

General Description

DNA pVR 1012-GP(S)
Local object
Created: 09/14/98 03:58PM
Last Modification Date: ? (no data)
length: 7073 bp
storage type: Basic
form: Circular
Comments

Restriction Map

Ball: 1 site TGGCCA / ACCGGT

BclI: 1 site TGATCA / ACTAGT

ClaI: 1 site ATCGAT / TAGCTA

DraIII: 1 site CACNNNGTG / GTGNNNCAC

HindIII: 1 site AAGCTT / TTCGAA

KasI: 1 site GGCGCC / CCGCGG

KpnI: 1 site GGTACC / CCATGG

NarI: 1 site GGCGCC / CCGCGG

PmlI: 1 site CACGTG / GTGCAC

PvuI: 1 site CGATCG / GCTAGC

SacII: 1 site CCGCGG / GGCGCC

SalI: 1 site GTCGAC / CAGCTG

XbaI: 1 site TCTAGA / AGATCT

XmnI: 1 site GAANNNNTTC / CTTNNNNAAG

NdeI: 2 sites CATATG / GTATAC

EcoRV: 3 sites GATATC / CTATAG

SphI: 3 sites GCATGC / CGTACG

NcoI: 4 sites CCATGG / GGTACC

BamHI: 6 sites GGATCC / CCTAGG

Functional Map

CDS (4 signals)

CMV IE 5' UT

Start: 886 End: 1129

CMV IE INT

Start: 1130 End: 1840

TbGH

Start: 4090 End: 4642

Kan r

Start: 6138 End: 6760 (Complementary)

Misc_feature (2 signals)

CMV enhancer

Start: 248 End: 885

GP(S)

Start: 1870 End: 4089

Annotations

```
1    TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

51   GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                          NdeI
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

                                                      BalI
201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                           NdeI
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                      NcoI
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA
```

```
 801 CGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     GCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT

 851 TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
     AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC

 901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
     TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA

                                      SacII
 951 TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
     AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT

1001 CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
     GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG

                                      SphI
1051 CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
     GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA

1101 TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
     AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC

1151 TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
     ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG

1201 TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
     ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT

1251 CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
     GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG

1301 ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
     TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA

1351 CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
     GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT

1401 GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
     CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA

1451 TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
     AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG

1501 AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
     TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC

1551 ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
     TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC

1601 TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
     ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC

1651 GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
     CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC
```

```
1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG

1751  TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
      ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

                                                  NcoI
1801  CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
      GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

                             SalI
                                  PmlI   BclI   EcoRV
      NcoI
1851  GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
      CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

                 SphI                               EcoRV
1901  CCGCTCTAGC TAGATGCATG CTCGAGCGGC CGCCAGTGTG ATGGATATCT
      GGCGAGATCG ATCTACGTAC GAGCTCGCCG GCGGTCACAC TACCTATAGA

                                NcoI
1951  GCAGAATTCT ATCTTCAGGA TCTCGCCATG GAGGGTCTTA GCCTACTCCA
      CGTCTTAAGA TAGAAGTCCT AGAGCGGTAC CTCCCAGAAT CGGATGAGGT

2001  ATTGCCCAGA GATAAATTTC GAAAAAGCTC TTTCTTTGTT TGGGTCATCA
      TAACGGGTCT CTATTTAAAG CTTTTTCGAG AAAGAAACAA ACCCAGTAGT

2051  TCTTATTTCA AAAGGCCTTT TCCATGCCTT TGGGTGTTGT GACCAACAGC
      AGAATAAAGT TTTCCGGAAA AGGTACGGAA ACCCACAACA CTGGTTGTCG

2101  ACTTTAGAAG TAACAGAGAT TGACCAGCTA GTCTGCAAGG ATCATCTTGC
      TGAAATCTTC ATTGTCTCTA ACTGGTCGAT CAGACGTTCC TAGTAGAACG

2151  ATCAACTGAC CAGCTGAAAT CAGTTGGTCT CAACCTCGAG GGGAGCGGAG
      TAGTTGACTG GTCGACTTTA GTCAACCAGA GTTGGAGCTC CCCTCGCCTC

           EcoRV
2201  TATCTACTGA TATCCCATCT GCGACAAAGC GTTGGGGCTT CAGATCTGGT
      ATAGATGACT ATAGGGTAGA CGCTGTTTCG CAACCCCGAA GTCTAGACCA

2251  GTGCCTCCCC AAGTGGTCAG CTATGAAGCA GGAGAATGGG CTGAAAATTG
      CACGGAGGGG TTCACCAGTC GATACTTCGT CCTCTTACCC GACTTTTAAC

2301  CTACAATCTT GAAATAAAGA AACCGGACGG GAGCGAATGC TTACCCCCAC
      GATGTTAGAA CTTTATTTCT TTGGCCTGCC CTCGCTTACG AATGGGGGTG

2351  CGCCGGATGG TGTCAGAGGC TTTCCAAGGT GCCGCTATGT TCACAAAGCC
      GCGGCCTACC ACAGTCTCCG AAAGGTTCCA CGGCGATACA AGTGTTTCGG

2401  CAAGGAACCG GGCCCTGCCC GGGTGACTAT GCCTTTCACA AGGATGGAGC
      GTTCCTTGGC CCGGGACGGG CCCACTGATA CGGAAAGTGT TCCTACCTCG

2451  TTTCTTCCTC TATGACAGGC TGGCTTCAAC TGTAATTTAC AGAGGAGTCA
      AAAGAAGGAG ATACTGTCCG ACCGAAGTTG ACATTAAATG TCTCCTCAGT
```

```
2501  ATTTTGCTGA GGGGGTAATC GCATTCTTGA TATTGGCTAA ACCAAAGGAA
      TAAAACGACT CCCCCATTAG CGTAAGAACT ATAACCGATT TGGTTTCCTT

2551  ACGTTCCTTC AATCACCCCC CATTCGAGAG GCAGCAAACT ACACTGAAAA
      TGCAAGGAAG TTAGTGGGGG GTAAGCTCTC CGTCGTTTGA TGTGACTTTT

2601  TACATCAAGT TACTATGCCA CATCCTACTT GGAGTACGAA ATCGAAAATT
      ATGTAGTTCA ATGATACGGT GTAGGATGAA CCTCATGCTT TAGCTTTTAA

2651  TTGGTGCTCA ACACTCCACG ACCCTTTTCA AAATTAACAA TAATACTTTT
      AACCACGAGT TGTGAGGTGC TGGGAAAAGT TTTAATTGTT ATTATGAAAA

2701  GTTCTTCTGG ACAGGCCCCA CACGCCTCAG TTCCTTTTCC AGCTGAATGA
      CAAGAAGACC TGTCCGGGGT GTGCGGAGTC AAGGAAAAGG TCGACTTACT

2751  TACCATTCAA CTTCACCAAC AGTTGAGCAA CACAACTGGG AAACTAATTT
      ATGGTAAGTT GAAGTGGTTG TCAACTCGTT GTGTTGACCC TTTGATTAAA

2801  GGACACTAGA TGCTAATATC AATGCTGATA TTGGTGAATG GGCTTTTTGG
      CCTGTGATCT ACGATTATAG TTACGACTAT AACCACTTAC CCGAAAAACC

2851  GAAAATAAAA AAATCTCTCC GAACAACTAC GTGGAGAAGA GCTGTCTTTC
      CTTTTATTTT TTTAGAGAGG CTTGTTGATG CACCTCTTCT CGACAGAAAG

2901  GAAACTTTAT CGCTCAACGA GACAGAAGAC GATGATGCGA CATCGTCGAG
      CTTTGAAATA GCGAGTTGCT CTGTCTTCTG CTACTACGCT GTAGCAGCTC

2951  AACTACAAAG GGAAGAATCT CCGACCGGGC CACCAGGAAG TATTCGGACC
      TTGATGTTTC CCTTCTTAGA GGCTGGCCCG GTGGTCCTTC ATAAGCCTGG

3001  TGGTTCCAAA GGATTCCCCT GGGATGGTTT CATTGCACGT ACCAGAAGGG
      ACCAAGGTTT CCTAAGGGGA CCCTACCAAA GTAACGTGCA TGGTCTTCCC

3051  GAAACAACAT TGCCGTCTCA GAATTCGACA GAAGGTCGAA GAGTAGATGT
      CTTTGTTGTA ACGGCAGAGT CTTAAGCTGT CTTCCAGCTT CTCATCTACA

3101  GAATACTCAG GAAACTATCA CAGAGACAAC TGCAACAATC ATAGGCACTA
      CTTATGAGTC CTTTGATAGT GTCTCTGTTG ACGTTGTTAG TATCCGTGAT

3151  ACGGTAACAA CATGCAGATC TCCACCATCG GGACAGGACT GAGCTCCAGC
      TGCCATTGTT GTACGTCTAG AGGTGGTAGC CCTGTCCTGA CTCGAGGTCG

                                 NcoI
3201  CAAATCCTGA GTTCCTCACC GACCATGGCA CCAAGCCCTG AGACTCAGAC
      GTTTAGGACT CAAGGAGTGG CTGGTACCGT GGTTCGGGAC TCTGAGTCTG

3251  CTCCACAACC TACACACCAA AACTACCAGT GATGACCACC GAGGAACCAA
      GAGGTGTTGG ATGTGTGGTT TTGATGGTCA CTACTGGTGG CTCCTTGGTT

3301  CAACACCACC GAGAAACTCT CCTGGCTCAA CAACAGAAGC ACCCACTCTC
      GTTGTGGTGG CTCTTTGAGA GGACCGAGTT GTTGTCTTCG TGGGTGAGAG

3351  ACCACCCCAG AGAATATAAC AACAGCGGTT AAAACTGTTT GGGCACAAGA
      TGGTGGGGTC TCTTATATTG TTGTCGCCAA TTTTGACAAA CCCGTGTTCT
```

```
3401  GTCCACAAGC AACGGTCTAA TAACTTCAAC AGTAACAGGT ATTCTTGGGA
      CAGGTGTTCG TTGCCAGATT ATTGAAGTTG TCATTGTCCA TAAGAACCCT

3451  GCCTTGGACT TCGAAAACGC AGCAGAAGAC AAGTTAACAC CAGGGCCACG
      CGGAACCTGA AGCTTTTGCG TCGTCTTCTG TTCAATTGTG GTCCCGGTGC

3501  GGTAAATGCA ATCCCAACTT ACACTACTGG ACTGCACAAG AACAACATAA
      CCATTTACGT TAGGGTTGAA TGTGATGACC TGACGTGTTC TTGTTGTATT

                         BamHI
3551  TGCTGCTGGG ATTGCCTGGA TCCCGTACTT TGGACCGGGT GCAGAAGGCA
      ACGACGACCC TAACGGACCT AGGGCATGAA ACCTGGCCCA CGTCTTCCGT

3601  TATACACTGA AGGCCTTATG CACAACCAAA ATGCCTTAGT CTGTGGACTC
      ATATGTGACT TCCGGAATAC GTGTTGGTTT TACGGAATCA GACACCTGAG

3651  AGACAACTTG CAAATGAAAC AACTCAAGCT CTGCAGCTTT TCTTAAGGGC
      TCTGTTGAAC GTTTACTTTG TTGAGTTCGA GACGTCGAAA AGAATTCCCG

3701  CACGACGGAG CTGCGGACAT ATACCATACT CAATAGGAAG GCCATAGATT
      GTGCTGCCTC GACGCCTGTA TATGGTATGA GTTATCCTTC CGGTATCTAA

                                     BamHI
3751  TCCTTCTGCG ACGATGGGGC GGGACATGTA GGATCCTGGG ACCAGATTGT
      AGGAAGACGC TGCTACCCCG CCCTGTACAT CCTAGGACCC TGGTCTAACA

3801  TGCATTGAGC CACATGATTG GACCAAAAAC ATCACTGATA AAATCAACCA
      ACGTAACTCG GTGTACTAAC CTGGTTTTTG TAGTGACTAT TTTAGTTGGT

3851  AATCATCCAT GATTTCATCG ACAACCCTTT ACCCAATCAG GATAATGATG
      TTAGTAGGTA CTAAAGTAGC TGTTGGGAAA TGGGTTAGTC CTATTACTAC

                                BamHI
3901  ATAATTGGTG GACGGGCTGG AGACAGTGGA TCCCTGCAGG AATAGGCATT
      TATTAACCAC CTGCCCGACC TCTGTCACCT AGGGACGTCC TTATCCGTAA

3951  ACTGGAATTA TTATTGCAAT CATTGCTCTT CTTTGCGTCT GCAAGCTGCT
      TGACCTTAAT AATAACGTTA GTAACGAGAA GAAACGCAGA CGTTCGACGA

                                                 BamHI
4001  TTGTTGAATA TCAGAATTCC AGCACTGGCG GCCGTTACTA GTGGATCCGA
      AACAACTTAT AGTCTTAAGG TCGTGACCGC CGGCAATGAT CACCTAGGCT

                              NarI
          BamHI     XbaI      KasI     BamHI
4051  GCTCGGATCC AAGCTCTAGA CCAGGCGCCT GGATCCAGAT CTGCTGTGCC
      CGAGCCTAGG TTCGAGATCT GGTCCGCGGA CCTAGGTCTA GACGACACGG

4101  TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA
      AAGATCAACG GTCGGTAGAC AACAAACGGG GAGGGGGCAC GGAAGGAACT

4151  CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT
      GGGACCTTCC ACGGTGAGGG TGACAGGAAA GGATTATTTT ACTCCTTTAA
```

```
4201  GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG
      CGTAGCGTAA CAGACTCATC CACAGTAAGA TAAGACCCCC CACCCCACCC

                                                 SphI
4251  GCAGCACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG
      CGTCGTGTCG TTCCCCCTCC TAACCCTTCT GTTATCGTCC GTACGACCCC

                     KpnI
4301  ATGCGGTGGG CTCTATGGGT ACCCAGGTGC TGAAGAATTG ACCCGGTTCC
      TACGCCACCC GAGATACCCA TGGGTCCACG ACTTCTTAAC TGGGCCAAGG

4351  TCCTGGGCCA GAAAGAAGCA GGCACATCCC CTTCTCTGTG ACACACCCTG
      AGGACCCGGT CTTTCTTCGT CCGTGTAGGG GAAGAGACAC TGTGTGGGAC

4401  TCCACGCCCC TGGTTCTTAG TTCCAGCCCC ACTCATAGGA CACTCATAGC
      AGGTGCGGGG ACCAAGAATC AAGGTCGGGG TGAGTATCCT GTGAGTATCG

4451  TCAGGAGGGC TCCGCCTTCA ATCCCACCCG CTAAAGTACT TGGAGCGGTC
      AGTCCTCCCG AGGCGGAAGT TAGGGTGGGC GATTTCATGA ACCTCGCCAG

4501  TCTCCCTCCC TCATCAGCCC ACCAAACCAA ACCTAGCCTC CAAGAGTGGG
      AGAGGGAGGG AGTAGTCGGG TGGTTTGGTT TGGATCGGAG GTTCTCACCC

4551  AAGAAATTAA AGCAAGATAG GCTATTAAGT GCAGAGGGAG AGAAAATGCC
      TTCTTTAATT TCGTTCTATC CGATAATTCA CGTCTCCCTC TCTTTTACGG

                                              XmnI
4601  TCCAACATGT GAGGAAGTAA TGAGAGAAAT CATAGAATTT CTTCCGCTTC
      AGGTTGTACA CTCCTTCATT ACTCTCTTTA GTATCTTAAA GAAGGCGAAG

4651  CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
      GAGCGAGTGA CTGAGCGACG CGAGCCAGCA AGCCGACGCC GCTCGCCATA

4701  CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC
      GTCGAGTGAG TTTCCGCCAT TATGCCAATA GGTGTCTTAG TCCCCTATTG

4751  GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA
      CGTCCTTTCT TGTACACTCG TTTTCCGGTC GTTTTCCGGT CCTTGGCATT

4801  AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC
      TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG GGACTGCTCG

4851  ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC GACAGGACTA
      TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG CTGTCCTGAT

4901  TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT
      ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG CGAGAGGACA

4951  TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
      AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG GGAAGCCCTT

5001  GCGTGGCGCT TTCTCAATGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG
      CGCACCGCGA AAGAGTTACG AGTGCGACAT CCATAGAGTC AAGCCACATC
```

5051 GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA
CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC AAGTCGGGCT

5101 CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC
GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG GGCCATTCTG

5151 ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT TAGCAGAGCG
TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA ATCGTCTCGC

5201 AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACTACGG
TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG GATTGATGCC

5251 CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC TTCGGTCAAT

5301 CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT
GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT TTGGTGGCGA

5351 GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA
CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG CGTCTTTTTT

5401 AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT
TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA CTGCGAGTCA

5451 GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG
CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA TAGTTTTTCC

5501 ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA
TAGAAGTGGA TCTAGGAAAA TTTAATTTTT ACTTCAAAAT TTAGTTAGAT

5551 AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
TTCATATATA CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC

5601 AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA
TCCGTGGATA GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT

5651 CTCCGGGGGG GGGGGGCGCT GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG
GAGGCCCCCC CCCCCCGCGA CTCCAGACGG AGCACTTCTT CCACAACGAC

5701 ACTCATACCA GGCCTGAATC GCCCCATCAT CCAGCCAGAA AGTGAGGGAG
TGAGTATGGT CCGGACTTAG CGGGGTAGTA GGTCGGTCTT TCACTCCCTC

5751 CCACGGTTGA TGAGAGCTTT GTTGTAGGTG GACCAGTTGG TGATTTTGAA
GGTGCCAACT ACTCTCGAAA CAACATCCAC CTGGTCAACC ACTAAAACTT

5801 CTTTTGCTTT GCCACGGAAC GGTCTGCGTT GTCGGGAAGA TGCGTGATCT
GAAAACGAAA CGGTGCCTTG CCAGACGCAA CAGCCCTTCT ACGCACTAGA

5851 GATCCTTCAA CTCAGCAAAA GTTCGATTTA TTCAACAAAG CCGCCGTCCC
CTAGGAAGTT GAGTCGTTTT CAAGCTAAAT AAGTTGTTTC GGCGGCAGGG

5901 GTCAAGTCAG CGTAATGCTC TGCCAGTGTT ACAACCAATT AACCAATTCT
CAGTTCAGTC GCATTACGAG ACGGTCACAA TGTTGGTTAA TTGGTTAAGA

5951 GATTAGAAAA ACTCATCGAG CATCAAATGA AACTGCAATT TATTCATATC
CTAATCTTTT TGAGTAGCTC GTAGTTTACT TTGACGTTAA ATAAGTATAG

```
6001  AGGATTATCA ATACCATATT TTTGAAAAAG CCGTTTCTGT AATGAAGGAG
      TCCTAATAGT TATGGTATAA AAACTTTTTC GGCAAAGACA TTACTTCCTC

6051  AAAACTCACC GAGGCAGTTC CATAGGATGG CAAGATCCTG GTATCGGTCT
      TTTTGAGTGG CTCCGTCAAG GTATCCTACC GTTCTAGGAC CATAGCCAGA

6101  GCGATTCCGA CTCGTCCAAC ATCAATACAA CCTATTAATT TCCCCTCGTC
      CGCTAAGGCT GAGCAGGTTG TAGTTATGTT GGATAATTAA AGGGGAGCAG

6151  AAAAATAAGG TTATCAAGTG AGAAATCACC ATGAGTGACG ACTGAATCCG
      TTTTTATTCC AATAGTTCAC TCTTTAGTGG TACTCACTGC TGACTTAGGC

                 HindIII
6201  GTGAGAATGG CAAAAGCTTA TGCATTTCTT TCCAGACTTG TTCAACAGGC
      CACTCTTACC GTTTTCGAAT ACGTAAAGAA AGGTCTGAAC AAGTTGTCCG

6251  CAGCCATTAC GCTCGTCATC AAAATCACTC GCATCAACCA AACCGTTATT
      GTCGGTAATG CGAGCAGTAG TTTTAGTGAG CGTAGTTGGT TTGGCAATAA

                                          PvuI
6301  CATTCGTGAT TGCGCCTGAG CGAGACGAAA TACGCGATCG CTGTTAAAAG
      GTAAGCACTA ACGCGGACTC GCTCTGCTTT ATGCGCTAGC GACAATTTTC

6351  GACAATTACA AACAGGAATC GAATGCAACC GGCGCAGGAA CACTGCCAGC
      CTGTTAATGT TTGTCCTTAG CTTACGTTGG CCGCGTCCTT GTGACGGTCG

6401  GCATCAACAA TATTTTCACC TGAATCAGGA TATTCTTCTA ATACCTGGAA
      CGTAGTTGTT ATAAAAGTGG ACTTAGTCCT ATAAGAAGAT TATGGACCTT

6451  TGCTGTTTTC CCGGGGATCG CAGTGGTGAG TAACCATGCA TCATCAGGAG
      ACGACAAAAG GGCCCCTAGC GTCACCACTC ATTGGTACGT AGTAGTCCTC

6501  TACGGATAAA ATGCTTGATG GTCGGAAGAG GCATAAATTC CGTCAGCCAG
      ATGCCTATTT TACGAACTAC CAGCCTTCTC CGTATTTAAG GCAGTCGGTC

6551  TTTAGTCTGA CCATCTCATC TGTAACATCA TTGGCAACGC TACCTTTGCC
      AAATCAGACT GGTAGAGTAG ACATTGTAGT AACCGTTGCG ATGGAAACGG

                                                     ClaI
6601  ATGTTTCAGA AACAACTCTG GCGCATCGGG CTTCCCATAC AATCGATAGA
      TACAAAGTCT TTGTTGAGAC CGCGTAGCCC GAAGGGTATG TTAGCTATCT

6651  TTGTCGCACC TGATTGCCCG ACATTATCGC GAGCCCATTT ATACCCATAT
      AACAGCGTGG ACTAACGGGC TGTAATAGCG CTCGGGTAAA TATGGGTATA

6701  AAATCAGCAT CCATGTTGGA ATTTAATCGC GGCCTCGAGC AAGACGTTTC
      TTTAGTCGTA GGTACAACCT TAAATTAGCG CCGGAGCTCG TTCTGCAAAG

6751  CCGTTGAATA TGGCTCATAA CACCCCTTGT ATTACTGTTT ATGTAAGCAG
      GGCAACTTAT ACCGAGTATT GTGGGGAACA TAATGACAAA TACATTCGTC

6801  ACAGTTTTAT TGTTCATGAT GATATATTTT TATCTTGTGC AATGTAACAT
      TGTCAAAATA ACAAGTACTA CTATATAAAA ATAGAACACG TTACATTGTA
```

```
                    DraIII
6851  CAGAGATTTT GAGACACAAC GTGGCTTTCC CCCCCCCCCC ATTATTGAAG
      GTCTCTAAAA CTCTGTGTTG CACCGAAAGG GGGGGGGGGG TAATAACTTC

6901  CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT
      GTAAATAGTC CCAATAACAG AGTACTCGCC TATGTATAAA CTTACATAAA

6951  AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA
      TCTTTTTATT TGTTTATCCC CAAGGCGCGT GTAAAGGGGC TTTTCACGGT

7001  CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG
      GGACTGCAGA TTCTTTGGTA ATAATAGTAC TGTAATTGGA TATTTTTATC

7051  GCGTATCACG AGGCCCTTTC GTC
      CGCATAGTGC TCCGGGAAAG CAG
```

SEQUENCE LISTING ID NO: 3

pVR 1012-GP(Z)

General Description

DNA pVR 1012-GP(Z)
Local object
Created: 09/15/98 05:06PM
Last Modification Date: ? (no data)
length: 7285 bp
storage type: Basic
form: Circular

Comments

Restriction Map

DraIII: 1 site CACNNNGTG
GTGNNNCAC

HindIII: 1 site AAGCTT
TTCGAA

HpaI: 1 site GTTAAC
CAATTG

KasI: 1 site GGCGCC
CCGCGG

NarI: 1 site GGCGCC
CCGCGG

NotI: 1 site GCGGCCGC
CGCCGGCG

PmlI: 1 site CACGTG
GTGCAC

PvuI: 1 site CGATCG
GCTAGC

SacII: 1 site CCGCGG
GGCGCC

XbaI: 1 site TCTAGA
AGATCT

XhoI: 1 site CTCGAG
GAGCTC

EcoRV: 2 sites GATATC
CTATAG

NcoI: 2 sites CCATGG
GGTACC

NdeI: 2 sites CATATG
GTATAC

SphI: 2 sites GCATGC
CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1860

TbGH
Start: 4302 End: 4854

Kan r
Start: 6350 End: 6972 (Complementary)

Misc_featur (2 signals)

CMV enhancer

Start: 248 End: 885

GP(Z)

Start: 1870 End: 4301

Annotations

```
  1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
    AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51 GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
    CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101 TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
    AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                       NdeI
                                       ----
151 CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
    GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
    GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251 TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
    AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
    AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351 AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
    TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401 ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
    TGTATTGAAT CCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451 CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
    GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501 CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
    GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                         NdeI
                         ----
551 GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
    CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
    ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                    NcoI
                                                    ----
651 ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
    TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

    NcoI
    -
701 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
    CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751 ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
    TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801 GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
    CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
```

```
851   TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
      AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC

901   AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
      TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA

                                          SacII
951   TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
      AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT

1001  CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
      GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG

                                          SphI
1051  CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
      GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA

1101  TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
      AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC

1151  TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
      ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG

1201  TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
      ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT

1251  CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
      GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG

1301  ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
      TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA

1351  CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
      GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT

1401  GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
      CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA

1451  TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
      AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG

1501  AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
      TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC

1551  ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
      TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC

1601  TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
      ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC

1651  GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
      CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC

1701  CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
      GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
```

HpaI

1751 TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

NcoI

1801 CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

NcoI PmlI EcoRVNotI

1851 GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

NarI

NotI XbaI KasI

1901 CCGCTCTAGA CCAGGCGCCT GGATCGATCC GCGATGAAGA TTAAGCCGAC
GGCGAGATCT GGTCCGCGGA CCTAGCTAGG CGCTACTTCT AATTCGGCTG

1951 AGTGAGCGTA ATCTTCATCT CTCTTAGATT ATTTGTTTTC CAGAGTAGGG
TCACTCGCAT TAGAAGTAGA GAGAATCTAA TAAACAAAAG GTCTCATCCC

2001 GTCGTCAGGT CCTTTTCAAT CGTGTAACCA AAATAAACTC CACTAGAAGG
CAGCAGTCCA GGAAAAGTTA GCACATTGGT TTTATTTGAG GTGATCTTCC

2051 ATATTGTGGG GCAACAACAC AATGGGCGTT ACAGGAATAT TGCAGTTACC
TATAACACCC CGTTGTTGTG TTACCCGCAA TGTCCTTATA ACGTCAATGG

2101 TCGTGATCGA TTCAAGAGGA CATCATTCTT TCTTTGGGTA ATTATCCTTT
AGCACTAGCT AAGTTCTCCT GTAGTAAGAA AGAAACCCAT TAATAGGAAA

2151 TCCAAAGAAC ATTTTCCATC CCACTTGGAG TCATCCACAA TAGCACATTA
AGGTTTCTTG TAAAAGGTAG GGTGAACCTC AGTAGGTGTT ATCGTGTAAT

2201 CAGGTTAGTG ATGTCGACAA ACTAGTTTGT CGTGACAAAC TGTCATCCAC
GTCCAATCAC TACAGCTGTT TGATCAAACA GCACTGTTTG ACAGTAGGTG

2251 AAATCAATTG AGATCAGTTG GACTGAATCT CGAAGGGAAT GGAGTGGCAA
TTTAGTTAAC TCTAGTCAAC CTGACTTAGA GCTTCCCTTA CCTCACCGTT

2301 CTGACGTGCC ATCTGCAACT AAAAGATGGG GCTTCAGGTC CGGTGTCCCA
GACTGCACGG TAGACGTTGA TTTTCTACCC CGAAGTCCAG GCCACAGGGT

2351 CCAAAGGTGG TCAATTATGA AGCTGGTGAA TGGGCTGAAA ACTGCTACAA
GGTTTCCACC AGTTAATACT TCGACCACTT ACCCGACTTT TGACGATGTT

2401 TCTTGAAATC AAAAAACCTG ACGGGAGTGA GTGTCTACCA GCAGCGCCAG
AGAACTTTAG TTTTTTGGAC TGCCCTCACT CACAGATGGT CGTCGCGGTC

2451 ACGGGATTCG GGGCTTCCCC CGGTGCCGGT ATGTGCACAA AGTATCAGGA
TGCCCTAAGC CCCGAAGGGG GCCACGGCCA TACACGTGTT TCATAGTCCT

2501 ACGGGACCGT GTGCCGGAGA CTTTGCCTTC CATAAAGAGG GTGCTTTCTT
TGCCCTGGCA CACGGCCTCT GAAACGGAAG GTATTTCTCC CACGAAAGAA

```
2551  CCTGTATGAT CGACTTGCTT CCACAGTTAT CTACCGAGGA ACGACTTTCG
      GGACATACTA GCTGAACGAA GGTGTCAATA GATGGCTCCT TGCTGAAAGC

2601  CTGAAGGTGT CGTTGCATTT CTGATACTGC CCCAAGCTAA GAAGGACTTC
      GACTTCCACA GCAACGTAAA GACTATGACG GGGTTCGATT CTTCCTGAAG

2651  TTCAGCTCAC ACCCCTTGAG AGAGCCGGTC AATGCAACGG AGGACCCGTC
      AAGTCGAGTG TGGGGAACTC TCTCGGCCAG TTACGTTGCC TCCTGGGCAG

                                  EcoRV
2701  TAGTGGCTAC TATTCTACCA CAATTAGATA TCAGGCTACC GGTTTTGGAA
      ATCACCGATG ATAAGATGGT GTTAATCTAT AGTCCGATGG CCAAAACCTT

2751  CCAATGAGAC AGAGTACTTG TTCGAGGTTG ACAATTTGAC CTACGTCCAA
      GGTTACTCTG TCTCATGAAC AAGCTCCAAC TGTTAAACTG GATGCAGGTT

2801  CTTGAATCAA GATTCACACC ACAGTTTCTG CTCCAGCTGA ATGAGACAAT
      GAACTTAGTT CTAAGTGTGG TGTCAAAGAC GAGGTCGACT TACTCTGTTA

2851  ATATACAAGT GGGAAAAGGA GCAATACCAC GGGAAAACTA ATTTGGAAGG
      TATATGTTCA CCCTTTTCCT CGTTATGGTG CCCTTTTGAT TAAACCTTCC

2901  TCAACCCCGA AATTGATACA ACAATCGGGG AGTGGGCCTT CTGGGAAACT
      AGTTGGGGCT TTAACTATGT TGTTAGCCCC TCACCCGGAA GACCCTTTGA

2951  AAAAAAAACC TCACTAGAAA AATTCGCAGT GAAGAGTTGT CTTTCACAGT
      TTTTTTTTGG AGTGATCTTT TTAAGCGTCA CTTCTCAACA GAAAGTGTCA

3001  TGTATCAAAC GGAGCCAAAA ACATCAGTGG TCAGAGTCCG GCGCGAACTT
      ACATAGTTTG CCTCGGTTTT TGTAGTCACC AGTCTCAGGC CGCGCTTGAA

3051  CTTCCGACCC AGGGACCAAC ACAACAACTG AAGACCACAA AATCATGGCT
      GAAGGCTGGG TCCCTGGTTG TGTTGTTGAC TTCTGGTGTT TTAGTACCGA

3101  TCAGAAAATT CCTCTGCAAT GGTTCAAGTG CACAGTCAAG GAAGGGAAGC
      AGTCTTTTAA GGAGACGTTA CCAAGTTCAC GTGTCAGTTC CTTCCCTTCG

3151  TGCAGTGTCG CATCTAACAA CCCTTGCCAC AATCTCCACG AGTCCCCAAT
      ACGTCACAGC GTAGATTGTT GGGAACGGTG TTAGAGGTGC TCAGGGGTTA

3201  CCCTCACAAC CAAACCAGGT CCGGACAACA GCACCCATAA TACACCCGTG
      GGGAGTGTTG GTTTGGTCCA GGCCTGTTGT CGTGGGTATT ATGTGGGCAC

3251  TATAAACTTG ACATCTCTGA GGCAACTCAA GTTGAACAAC ATCACCGCAG
      ATATTTGAAC TGTAGAGACT CCGTTGAGTT CAACTTGTTG TAGTGGCGTC

3301  AACAGACAAC GACAGCACAG CCTCCGACAC TCCCTCTGCC ACGACCGCAG
      TTGTCTGTTG CTGTCGTGTC GGAGGCTGTG AGGGAGACGG TGCTGGCGTC

3351  CCGGACCCCC AAAAGCAGAG AACACCAACA CGAGCAAGAG CACTGACTTC
      GGCCTGGGGG TTTTCGTCTC TTGTGGTTGT GCTCGTTCTC GTGACTGAAG

3401  CTGGACCCCG CCACCACAAC AAGTCCCCAA AACCACAGCG AGACCGCTGG
      GACCTGGGGC GGTGGTGTTG TTCAGGGGTT TTGGTGTCGC TCTGGCGACC
```

```
3451 CAACAACAAC ACTCATCACC AAGATACCGG AGAAGAGAGT GCCAGCAGCG
     GTTGTTGTTG TGAGTAGTGG TTCTATGGCC TCTTCTCTCA CGGTCGTCGC

3501 GGAAGCTAGG CTTAATTACC AATACTATTG CTGGAGTCGC AGGACTGATC
     CCTTCGATCC GAATTAATGG TTATGATAAC GACCTCAGCG TCCTGACTAG

3551 ACAGGCGGGA GAAGAACTCG AAGAGAAGCA ATTGTCAATG CTCAACCCAA
     TGTCCGCCCT CTTCTTGAGC TTCTCTTCGT TAACAGTTAC GAGTTGGGTT

3601 ATGCAACCCT AATTTACATT ACTGGACTAC TCAGGATGAA GGTGCTGCAA
     TACGTTGGGA TTAAATGTAA TGACCTGATG AGTCCTACTT CCACGACGTT

3651 TCGGACTGGC CTGGATACCA TATTTCGGGC CAGCAGCCGA GGGAATTTAC
     AGCCTGACCG GACCTATGGT ATAAAGCCCG GTCGTCGGCT CCCTTAAATG

3701 ATAGAGGGCC TAATGCACAA TCAAGATGGT TTAATCTGTG GGTTGAGACA
     TATCTCCCGG ATTACGTGTT AGTTCTACCA AATTAGACAC CCAACTCTGT

3751 GCTGGCCAAC GAGACGACTC AAGCTCTTCA ACTGTTCCTG AGAGCCACAA
     CGACCGGTTG CTCTGCTGAG TTCGAGAAGT TGACAAGGAC TCTCGGTGTT

3801 CTGAGCTACG CACCTTTTCA ATCCTCAACC GTAAGGCAAT TGATTTCTTG
     GACTCGATGC GTGGAAAAGT TAGGAGTTGG CATTCCGTTA ACTAAAGAAC

3851 CTGCAGCGAT GGGGCGGCAC ATGCCACATT CTGGGACCGG ACTGCTGTAT
     GACGTCGCTA CCCCGCCGTG TACGGTGTAA GACCCTGGCC TGACGACATA

3901 CGAACCACAT GATTGGACCA AGAACATAAC AGACAAAATT GATCAGATTA
     GCTTGGTGTA CTAACCTGGT TCTTGTATTG TCTGTTTTAA CTAGTCTAAT

3951 TTCATGATTT TGTTGATAAA ACCCTTCCGG ACCAGGGGGA CAATGACAAT
     AAGTACTAAA ACAACTATTT TGGGAAGGCC TGGTCCCCCT GTTACTGTTA

4001 TGGTGGACAG GATGGAGACA ATGGATACCG GCAGGTATTG GAGTTACAGG
     ACCACCTGTC CTACCTCTGT TACCTATGGC CGTCCATAAC CTCAATGTCC

4051 CGTTATAATT GCAGTTATCG CTTTATTCTG TATATGCAAA TTTGTCTTTT
     GCAATATTAA CGTCAATAGC GAAATAAGAC ATATACGTTT AAACAGAAAA

4101 AGTTTTTCTT CAGATTGCTT CATGGAAAAG CTCAGCCTCA AATCAATGAA
     TCAAAAAGAA GTCTAACGAA GTACCTTTTC GAGTCGGAGT TTAGTTACTT

4151 ACCAGGATTT AATTATATGG ATTACTTGAA TCTAAGATTA CTTGACAAAT
     TGGTCCTAAA TTAATATACC TAATGAACTT AGATTCTAAT GAACTGTTTA

4201 GATAATATAA TACACTGGAG CTTTAAACAT AGCCAATGTG ATTCTAACTC
     CTATTATATT ATGTGACCTC GAAATTTGTA TCGGTTACAC TAAGATTGAG

4251 CTTTAAACTC ACAGTTAATC ATAAACAAGG TTTGGTACCG AGCTCGAATT
     GAAATTTGAG TGTCAATTAG TATTTGTTCC AAACCATGGC TCGAGCTTAA

4301 ATCTGCTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG
     TAGACGACAC GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC

4351 TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA
     ACGGAAGGAA CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT
```

```
4401  AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG
      TTACTCCTTT AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC

4451  GGGTGGGGTG GGGCAGCACA GCAAGGGGGA GGATTGGGAA GACAATAGCA
      CCCACCCCAC CCCGTCGTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT

       SphI
4501  GGCATGCTGG GGATGCGGTG GGCTCTATGG GTACCCAGGT GCTGAAGAAT
      CCGTACGACC CCTACGCCAC CCGAGATACC CATGGGTCCA CGACTTCTTA

4551  TGACCCGGTT CCTCCTGGGC CAGAAAGAAG CAGGCACATC CCCTTCTCTG
      ACTGGGCCAA GGAGGACCCG GTCTTTCTTC GTCCGTGTAG GGGAAGAGAC

4601  TGACACACCC TGTCCACGCC CCTGGTTCTT AGTTCCAGCC CCACTCATAG
      ACTGTGTGGG ACAGGTGCGG GGACCAAGAA TCAAGGTCGG GGTGAGTATC

4651  GACACTCATA GCTCAGGAGG GCTCCGCCTT CAATCCCACC CGCTAAAGTA
      CTGTGAGTAT CGAGTCCTCC CGAGGCGGAA GTTAGGGTGG GCGATTTCAT

4701  CTTGGAGCGG TCTCTCCCTC CCTCATCAGC CCACCAAACC AAACCTAGCC
      GAACCTCGCC AGAGAGGGAG GGAGTAGTCG GGTGGTTTGG TTTGGATCGG

4751  TCCAAGAGTG GGAAGAAATT AAAGCAAGAT AGGCTATTAA GTGCAGAGGG
      AGGTTCTCAC CCTTCTTTAA TTTCGTTCTA TCCGATAATT CACGTCTCCC

4801  AGAGAAAATG CCTCCAACAT GTGAGGAAGT AATGAGAGAA ATCATAGAAT
      TCTCTTTTAC GGAGGTTGTA CACTCCTTCA TTACTCTCTT TAGTATCTTA

4851  TTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC
      AAGAAGGCGA AGGAGCGAGT GACTGAGCGA CGCGAGCCAG CAAGCCGACG

4901  GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA
      CCGCTCGCCA TAGTCGAGTG AGTTTCCGCC ATTATGCCAA TAGGTGTCTT

4951  TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
      AGTCCCCTAT TGCGTCCTTT CTTGTACACT CGTTTTCCGG TCGTTTTCCG

5001  CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC
      GTCCTTGGCA TTTTTCCGGC GCAACGACCG CAAAAAGGTA TCCGAGGCGG

5051  CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC
      GGGGACTGCT CGTAGTGTTT TTAGCTGCGA GTTCAGTCTC CACCGCTTTG

5101  CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT
      GGCTGTCCTG ATATTTCTAT GGTCCGCAAA GGGGGACCTT CGAGGGAGCA

5151  GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC
      CGCGAGAGGA CAAGGCTGGG ACGGCGAATG GCCTATGGAC AGGCGGAAAG

5201  TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG TAGGTATCTC
      AGGGAAGCCC TTCGCACCGC GAAAGAGTTA CGAGTGCGAC ATCCATAGAG

5251  AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
      TCAAGCCACA TCCAGCAAGC GAGGTTCGAC CCGACACACG TGCTTGGGGG
```

5301 CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA
GCAAGTCGGG CTGGCGACGC GGAATAGGCC ATTGATAGCA GAACTCAGGT

5351 ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG
TGGGCCATTC TGTGCTGAAT AGCGGTGACC GTCGTCGGTG ACCATTGTCC

5401 ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG
TAATCGTCTC GCTCCATACA TCCGCCACGA TGTCTCAAGA ACTTCACCAC

5451 GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC
CGGATTGATG CCGATGTGAT CTTCCTGTCA TAAACCATAG ACGCGAGACG

5501 TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA
ACTTCGGTCA ATGGAAGCCT TTTTCTCAAC CATCGAGAAC TAGGCCGTTT

5551 CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
GTTTGGTGGC GACCATCGCC ACCAAAAAAA CAAACGTTCG TCGTCTAATG

5601 GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT
CGCGTCTTTT TTTCCTAGAG TTCTTCTAGG AAACTAGAAA AGATGCCCCA

5651 CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA
GACTGCGAGT CACCTTGCTT TTGAGTGCAA TTCCCTAAAA CCAGTACTCT

5701 TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT
AATAGTTTTT CCTAGAAGTG GATCTAGGAA AATTTAATTT TTACTTCAAA

5751 TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
ATTTAGTTAG ATTTCATATA TACTCATTTG AACCAGACTG TCAATGGTTA

5801 GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC
CGAATTAGTC ACTCCGTGGA TAGAGTCGCT AGACAGATAA AGCAAGTAGG

5851 ATAGTTGCCT GACTCCGGGG GGGGGGGGCG CTGAGGTCTG CCTCGTGAAG
TATCAACGGA CTGAGGCCCC CCCCCCCCGC GACTCCAGAC GGAGCACTTC

5901 AAGGTGTTGC TGACTCATAC CAGGCCTGAA TCGCCCCATC ATCCAGCCAG
TTCCACAACG ACTGAGTATG GTCCGGACTT AGCGGGGTAG TAGGTCGGTC

5951 AAAGTGAGGG AGCCACGGTT GATGAGAGCT TTGTTGTAGG TGGACCAGTT
TTTCACTCCC TCGGTGCCAA CTACTCTCGA AACAACATCC ACCTGGTCAA

6001 GGTGATTTTG AACTTTTGCT TTGCCACGGA ACGGTCTGCG TTGTCGGGAA
CCACTAAAAC TTGAAAACGA AACGGTGCCT TGCCAGACGC AACAGCCCTT

6051 GATGCGTGAT CTGATCCTTC AACTCAGCAA AAGTTCGATT TATTCAACAA
CTACGCACTA GACTAGGAAG TTGAGTCGTT TTCAAGCTAA ATAAGTTGTT

6101 AGCCGCCGTC CCGTCAAGTC AGCGTAATGC TCTGCCAGTG TTACAACCAA
TCGGCGGCAG GGCAGTTCAG TCGCATTACG AGACGGTCAC AATGTTGGTT

6151 TTAACCAATT CTGATTAGAA AAACTCATCG AGCATCAAAT GAAACTGCAA
AATTGGTTAA GACTAATCTT TTTGAGTAGC TCGTAGTTTA CTTTGACGTT

6201 TTTATTCATA TCAGGATTAT CAATACCATA TTTTTGAAAA AGCCGTTTCT
AAATAAGTAT AGTCCTAATA GTTATGGTAT AAAAACTTTT TCGGCAAAGA

```
6251  GTAATGAAGG AGAAAACTCA CCGAGGCAGT TCCATAGGAT GGCAAGATCC
      CATTACTTCC TCTTTTGAGT GGCTCCGTCA AGGTATCCTA CCGTTCTAGG

6301  TGGTATCGGT CTGCGATTCC GACTCGTCCA ACATCAATAC AACCTATTAA
      ACCATAGCCA GACGCTAAGG CTGAGCAGGT TGTAGTTATG TTGGATAATT

6351  TTTCCCCTCG TCAAAAATAA GGTTATCAAG TGAGAAATCA CCATGAGTGA
      AAAGGGGAGC AGTTTTTATT CCAATAGTTC ACTCTTTAGT GGTACTCACT

                                HindIII
6401  CGACTGAATC CGGTGAGAAT GGCAAAAGCT TATGCATTTC TTTCCAGACT
      GCTGACTTAG GCCACTCTTA CCGTTTTCGA ATACGTAAAG AAAGGTCTGA

6451  TGTTCAACAG GCCAGCCATT ACGCTCGTCA TCAAAATCAC TCGCATCAAC
      ACAAGTTGTC CGGTCGGTAA TGCGAGCAGT AGTTTTAGTG AGCGTAGTTG

                                                       PvuI
6501  CAAACCGTTA TTCATTCGTG ATTGCGCCTG AGCGAGACGA AATACGCGAT
      GTTTGGCAAT AAGTAAGCAC TAACGCGGAC TCGCTCTGCT TTATGCGCTA

      PvuI
6551  CGCTGTTAAA AGGACAATTA CAAACAGGAA TCGAATGCAA CCGGCGCAGG
      GCGACAATTT TCCTGTTAAT GTTTGTCCTT AGCTTACGTT GGCCGCGTCC

6601  AACACTGCCA GCGCATCAAC AATATTTTCA CCTGAATCAG GATATTCTTC
      TTGTGACGGT CGCGTAGTTG TTATAAAAGT GGACTTAGTC CTATAAGAAG

6651  TAATACCTGG AATGCTGTTT TCCCGGGGAT CGCAGTGGTG AGTAACCATG
      ATTATGGACC TTACGACAAA AGGGCCCCTA GCGTCACCAC TCATTGGTAC

6701  CATCATCAGG AGTACGGATA AAATGCTTGA TGGTCGGAAG AGGCATAAAT
      GTAGTAGTCC TCATGCCTAT TTTACGAACT ACCAGCCTTC TCCGTATTTA

6751  TCCGTCAGCC AGTTTAGTCT GACCATCTCA TCTGTAACAT CATTGGCAAC
      AGGCAGTCGG TCAAATCAGA CTGGTAGAGT AGACATTGTA GTAACCGTTG

6801  GCTACCTTTG CCATGTTTCA GAAACAACTC TGGCGCATCG GGCTTCCCAT
      CGATGGAAAC GGTACAAAGT CTTTGTTGAG ACCGCGTAGC CCGAAGGGTA

6851  ACAATCGATA GATTGTCGCA CCTGATTGCC CGACATTATC GCGAGCCCAT
      TGTTAGCTAT CTAACAGCGT GGACTAACGG GCTGTAATAG CGCTCGGGTA

                                                       XhoI
6901  TTATACCCAT ATAAATCAGC ATCCATGTTG GAATTTAATC GCGGCCTCGA
      AATATGGGTA TATTTAGTCG TAGGTACAAC CTTAAATTAG CGCCGGAGCT

      XhoI
6951  GCAAGACGTT TCCCGTTGAA TATGGCTCAT AACACCCCTT GTATTACTGT
      CGTTCTGCAA AGGGCAACTT ATACCGAGTA TTGTGGGGAA CATAATGACA

7001  TTATGTAAGC AGACAGTTTT ATTGTTCATG ATGATATATT TTTATCTTGT
      AATACATTCG TCTGTCAAAA TAACAAGTAC TACTATATAA AAATAGAACA
```

```
                                DraIII
                              ----------
7051  GCAATGTAAC ATCAGAGATT TTGAGACACA ACGTGGCTTT CCCCCCCCCC
      CGTTACATTG TAGTCTCTAA AACTCTGTGT TGCACCGAAA GGGGGGGGGG
...........................................................
7101  CCATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT
      GGTAATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA
...........................................................
7151  TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC
      AACTTACATA AATCTTTTTA TTTGTTTATC CCCAAGGCGC GTGTAAAGGG
...........................................................
7201  CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAC
      GCTTTTCACG GTGGACTGCA GATTCTTTGG TAATAATAGT ACTGTAATTG
...........................................................
7251  CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTC
      GATATTTTTA TCCGCATAGT GCTCCGGGAA AGCAG
...........................................................
```

pVR 1012-SCP(Z)

General Description

DNA pVR 1012-SCP(Z)
Local object
Created: 09/14/98 04:29PM
Last Modified: 09/15/98 04:50PM
length: 7272 bp
storage type: Basic
form: Circular

Comments

Restriction Map

DraIII: 1 site	CACNNNGTG GTGNNNCAC
HindIII: 1 site	AAGCTT TTCGAA
HpaI: 1 site	GTTAAC CAATTG
KpnI: 1 site	GGTACC CCATGG
NotI: 1 site	GCGGCCGC CGCCGGCG
PmlI: 1 site	CACGTG GTGCAC
PvuI: 1 site	CGATCG GCTAGC
SacII: 1 site	CCGCGG GGCGCC
XbaI: 1 site	TCTAGA AGATCT
XhoI: 1 site	CTCGAG GAGCTC
EcoRV: 2 sites	GATATC CTATAG
NcoI: 2 sites	CCATGG GGTACC
NdeI: 2 sites	CATATG GTATAC
SphI: 2 sites	GCATGC CGTACG

Functional Map

CDS (4 signals)

CMV IE 5' UT
Start: 886 End: 1129

CMV IE INT
Start: 1130 End: 1840

TbGH
Start: 4289 End: 4861

Kan r
Start: 6337 End: 6959 (Complementary)

Misc_feature (2 signals)

CMV enhancer

Start: 248 End: 885

SGP(Z)

Start: 1870 End: 4288

Annotations

```
  1  TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
     AGCGCGCAAA GCCACTACTG CCACTTTTGG AGACTGTGTA CGTCGAGGGC

 51  GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
     CTCTGCCAGT GTCGAACAGA CATTCGCCTA CGGCCCTCGT CTGTTCGGGC

101  TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG
     AGTCCCGCGC AGTCGCCCAC AACCGCCCAC AGCCCCGACC GAATTGATAC

                                        NdeI
151  CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
     GCCGTAGTCT CGTCTAACAT GACTCTCACG TGGTATACGC CACACTTTAT

201  CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
     GGCGTGTCTA CGCATTCCTC TTTTATGGCG TAGTCTAACC GATAACCGGT

251  TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
     AACGTATGCA ACATAGGTAT AGTATTATAC ATGTAAATAT AACCGAGTAC

301  TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
     AGGTTGTAAT GGCGGTACAA CTGTAACTAA TAACTGATCA ATAATTATCA

351  AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
     TTAGTTAATG CCCCAGTAAT CAAGTATCGG GTATATACCT CAAGGCGCAA

401  ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
     TGTATTGAAT GCCATTTACC GGGCGGACCG ACTGGCGGGT TGCTGGGGGC

451  CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
     GGGTAACTGC AGTTATTACT GCATACAAGG GTATCATTGC GGTTATCCCT

501  CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
     GAAAGGTAAC TGCAGTTACC CACCTCATAA ATGCCATTTG ACGGGTGAAC

                           NdeI
551  GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
     CGTCATGTAG TTCACATAGT ATACGGTTCA TGCGGGGGAT AACTGCAGTT

601  TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
     ACTGCCATTT ACCGGGCGGA CCGTAATACG GGTCATGTAC TGGAATACCC

                                                    NcoI
651  ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
     TGAAAGGATG AACCGTCATG TAGATGCATA ATCAGTAGCG ATAATGGTAC

     NcoI
701  GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
     CACTACGCCA AAACCGTCAT GTAGTTACCC GCACCTATCG CCAAACTGAG

751  ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
     TGCCCCTAAA GGTTCAGAGG TGGGGTAACT GCAGTTACCC TCAAACAAAA

801  GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
     CCGTGGTTTT AGTTGCCCTG AAAGGTTTTA CAGCATTGTT GAGGCGGGGT
```

```
 851 TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
     AACTGCGTTT ACCCGCCATC CGCACATGCC ACCCTCCAGA TATATTCGTC

 901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
     TCGAGCAAAT CACTTGGCAG TCTAGCGGAC CTCTGCGGTA GGTGCGACAA

                                      SacII
 951 TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG CGGCCGGGAA
     AACTGGAGGT ATCTTCTGTG GCCCTGGCTA GGTCGGAGGC GCCGGCCCTT

1001 CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC
     GCCACGTAAC CTTGCGCCTA AGGGGCACGG TTCTCACTGC ATTCATGGCG

                                      SphI
1051 CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT
     GATATCTGAG ATATCCGTGT GGGGAAACCG AGAATACGTA CGATATGACA

1101 TTTTGGCTTG GGGCCTATAC ACCCCCGCTT CCTTATGCTA TAGGTGATGG
     AAAACCGAAC CCCGGATATG TGGGGGCGAA GGAATACGAT ATCCACTACC

1151 TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG ACCACTCCCC
     ATATCGAATC GGATATCCAC ACCCAATAAC TGGTAATAAC TGGTGAGGGG

1201 TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA
     ATAACCACTG CTATGAAAGG TAATGATTAG GTATTGTACC GAGAAACGGT

1251 CAACTATCTC TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC
     GTTGATAGAG ATAACCGATA TACGGTTATG AGACAGGAAG TCTCTGACTG

1301 ACGGACTCTG TATTTTTACA GGATGGGGTC CCATTTATTA TTTACAAATT
     TGCCTGAGAC ATAAAAATGT CCTACCCCAG GGTAAATAAT AAATGTTTAA

1351 CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA
     GTGTATATGT TGTTGCGGCA GGGGGCACGG GCGTCAAAAA TAATTTGTAT

1401 GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT
     CGCACCCTAG AGGTGCGCTT AGAGCCCATG CACAAGGCCT GTACCCGAGA

1451 TCTCCGGTAG CGGCGGAGCT TCCACATCCG AGCCCTGGTC CCATGCCTCC
     AGAGGCCATC GCCGCCTCGA AGGTGTAGGC TCGGGACCAG GGTACGGAGG

1501 AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG TGGAGGCCAG
     TCGCCGAGTA CCAGCGAGCC GTCGAGGAAC GAGGATTGTC ACCTCCGGTC

1551 ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG
     TGAATCCGTG TCGTGTTACG GGTGGTGGTG GTCACACGGC GTGTTCCGGC

1601 TGGCGGTAGG GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG
     ACCGCCATCC CATACACAGA CTTTTACTCG CACCTCTAAC CCGAGCGTGC

1651 GCTGACGCAG ATGGAAGACT TAAGGCAGCG GCAGAAGAAG ATGCAGGCAG
     CGACTGCGTC TACCTTCTGA ATTCCGTCGC CGTCTTCTTC TACGTCCGTC

1701 CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC
     GACTCAACAA CATAAGACTA TTCTCAGTCT CCATTGAGGG CAACGCCACG
```

```
     HpaI
1751 TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG
     ACAATTGCCA CCTCCCGTCA CATCAGACTC GTCATGAGCA ACGACGGCGC

                                                     NcoI
1801 CGCGCCACCA GACATAATAG CTGACAGACT AACAGACTGT TCCTTTCCAT
     GCGCGGTGGT CTGTATTATC GACTGTCTGA TTGTCTGACA AGGAAAGGTA

     NcoI                              PmlI       EcoRVNotI
1851 GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA GATATCGCGG
     CCCAGAAAAG ACGTCAGTGG CAGCAGCTGT GCACACTAGT CTATAGCGCC

     NotI XbaI
1901 CCGCTCTAGA CCAGGCGCCT GGATCGAATT GATGAAGATT AAGCCGACAG
     GGCGAGATCT GGTCCGCGGA CCTAGCTTAA CTACTTCTAA TTCGGCTGTC

1951 TGAGCGTAAT CTTCATCTCT CTTAGATTAT TTGTTTTCCA GAGTAGGGGT
     ACTCGCATTA GAAGTAGAGA GAATCTAATA AACAAAAGGT CTCATCCCCA

2001 CGTCAGGTCC TTTTCAATCG TGTAACCAAA ATAAACTCCA CTAGAAGGAT
     GCAGTCCAGG AAAAGTTAGC ACATTGGTTT TATTTGAGGT GATCTTCCTA

2051 ATTGTGGGGC AACAACACAA TGGGCGTTAC AGGAATATTG CAGTTACCTC
     TAACACCCCG TTGTTGTGTT ACCCGCAATG TCCTTATAAC GTCAATGGAG

2101 GTGATCGATT CAAGAGGACA TCATTCTTTC TTTGGGTAAT TATCCTTTTC
     CACTAGCTAA GTTCTCCTGT AGTAAGAAAG AAACCCATTA ATAGGAAAAG

2151 CAAAGAACAT TTTCCATCCC ACTTGGAGTC ATCCACAATA GCACATTACA
     GTTTCTTGTA AAAGGTAGGG TGAACCTCAG TAGGTGTTAT CGTGTAATGT

2201 GGTTAGTGAT GTCGACAAAC TAGTTTGTCG TGACAAACTG TCATCCACAA
     CCAATCACTA CAGCTGTTTG ATCAAACAGC ACTGTTTGAC AGTAGGTGTT

2251 ATCAATTGAG ATCAGTTGGA CTGAATCTCG AAGGGAATGG AGTGGCAACT
     TAGTTAACTC TAGTCAACCT GACTTAGAGC TTCCCTTACC TCACCGTTGA

2301 GACGTGCCAT CTGCAACTAA AAGATGGGGC TTCAGGTCCG GTGTCCCACC
     CTGCACGGTA GACGTTGATT TTCTACCCCG AAGTCCAGGC CACAGGGTGG

2351 AAAGGTGGTC AATTATGAAG CTGGTGAATG GGCTGAAAAC TGCTACAATC
     TTTCCACCAG TTAATACTTC GACCACTTAC CCGACTTTTG ACGATGTTAG

2401 TTGAAATCAA AAAACCTGAC GGGAGTGAGT GTCTACCAGC AGCGCCAGAC
     AACTTTAGTT TTTTGGACTG CCCTCACTCA CAGATGGTCG TCGCGGTCTG

2451 GGGATTCGGG GCTTCCCCCG GTGCCGGTAT GTGCACAAAG TATCAGGAAC
     CCCTAAGCCC CGAAGGGGGC CACGGCCATA CACGTGTTTC ATAGTCCTTG

2501 GGGACCGTGT GCCGGAGACT TTGCCTTCCA TAAAGAGGGT GCTTTCTTCC
     CCCTGGCACA CGGCCTCTGA AACGGAAGGT ATTTCTCCCA CGAAAGAAGG

2551 TGTATGATCG ACTTGCTTCC ACAGTTATCT ACCGAGGAAC GACTTTCGCT
     ACATACTAGC TGAACGAAGG TGTCAATAGA TGGCTCCTTG CTGAAAGCGA
```

2601 CAAGGTGTCG TTGCATTTCT GATACTGCCC CAAGCTAAGA AGGACTTCTT
GTTCCACAGC AACGTAAAGA CTATGACGGG GTTCGATTCT TCCTGAAGAA

2651 CAGCTCACAC CCCTTGAGAG AGCCGGTCAA TGCAACGGAG GACCCGTCTA
GTCGAGTGTG GGGAACTCTC TCGGCCAGTT ACGTTGCCTC CTGGGCAGAT

EcoRV

2701 GTGGCTACTA TTCTACCACA ATTAGATATC AGGCTACCGG TTTTGGAACC
CACCGATGAT AAGATGGTGT TAATCTATAG TCCGATGGCC AAAACCTTGG

2751 AATGAGACAG AGTACTTGTT CGAGGTTGAC AATTTGACCT ACGTCCAACT
TTACTCTGTC TCATGAACAA GCTCCAACTG TTAAACTGGA TGCAGGTTGA

2801 TGAATCAAGA TTCACACCAC AGTTTCTGCT CCAGCTGAAT GAGACAATAT
ACTTAGTTCT AAGTGTGGTG TCAAAGACGA GGTCGACTTA CTCTGTTATA

2851 ATACAAGTGG GAAAAGGAGC AATACCACGG GAAAACTAAT TTGGAAGGTC
TATGTTCACC CTTTTCCTCG TTATGGTGCC CTTTTGATTA AACCTTCCAG

2901 AACCCCGAAA TTGATACAAC AATCGGGGAG TGGGCCTTCT GGGAAACTAA
TTGGGGCTTT AACTATGTTG TTAGCCCCTC ACCCGGAAGA CCCTTTGATT

2951 AAAAACCTCA CTAGAAAAAT TCGCAGTGAA GAGTTGTCTT TCACAGTTGT
TTTTTGGAGT GATCTTTTTA AGCGTCACTT CTCAACAGAA AGTGTCAACA

3001 ATCAAACGGA GCCAAAAACA TCAGTGGTCA GAGTCCGGCG CGAACTTCTT
TAGTTTGCCT CGGTTTTTGT AGTCACCAGT CTCAGGCCGC GCTTGAAGAA

3051 CCGACCCAGG GACCAACACA ACAACTGAAG ACCACAAAAT CATGGCTTCA
GGCTGGGTCC CTGGTTGTGT TGTTGACTTC TGGTGTTTTA GTACCGAAGT

3101 GAAAATTCCT CTGCAATGGT TCAAGTGCAC AGTCAAGGAA GGGAAGCTGC
CTTTTAAGGA GACGTTACCA AGTTCACGTG TCAGTTCCTT CCCTTCGACG

3151 AGTGTCGCAT CTAACAACCC TTGCCACAAT CTCCACGAGT CCCCAATCCC
TCACAGCGTA GATTGTTGGG AACGGTGTTA GAGGTGCTCA GGGGTTAGGG

3201 TCACAACCAA ACCAGGTCCG GACAACAGCA CCCATAATAC ACCCGTGTAT
AGTGTTGGTT TGGTCCAGGC CTGTTGTCGT GGGTATTATG TGGGCACATA

3251 AAACTTGACA TCTCTGAGGC AACTCAAGTT GAACAACATC ACCGCAGAAC
TTTGAACTGT AGAGACTCCG TTGAGTTCAA CTTGTTGTAG TGGCGTCTTG

3301 AGACAACGAC AGCACAGCCT CCGACACTCC CTCTGCCACG ACCGCAGCCG
TCTGTTGCTG TCGTGTCGGA GGCTGTGAGG GAGACGGTGC TGGCGTCGGC

3351 GACCCCCAAA AGCAGAGAAC ACCAACACGA GCAAGAGCAC TGACTTCCTG
CTGGGGGTTT TCGTCTCTTG TGGTTGTGCT CGTTCTCGTG ACTGAAGGAC

3401 GACCCCGCCA CCACAACAAG TCCCCAAAAC CACAGCGAGA CCGCTGGCAA
CTGGGGCGGT GGTGTTGTTC AGGGGTTTTG GTGTCGCTCT GGCGACCGTT

3451 CAACAACACT CATCACCAAG ATACCGGAGA AGAGAGTGCC AGCAGCGGGA
GTTGTTGTGA GTAGTGGTTC TATGGCCTCT TCTCTCACGG TCGTCGCCCT

```
3501  AGCTAGGCTT AATTACCAAT ACTATTGCTG GAGTCGCAGG ACTGATCACA
      TCGATCCGAA TTAATGGTTA TGATAACGAC CTCAGCGTCC TGACTAGTGT

3551  GGCGGGAGAA GAACTCGAAG AGAAGCAATT GTCAATGCTC AACCCAAATG
      CCGCCCTCTT CTTGAGCTTC TCTTCGTTAA CAGTTACGAG TTGGGTTTAC

3601  CAACCCTAAT TTACATTACT GGACTACTCA GGATGAAGGT GCTGCAATCG
      GTTGGGATTA AATGTAATGA CCTGATGAGT CCTACTTCCA CGACGTTAGC

3651  GACTGGCCTG GATACCATAT TTCGGGCCAG CAGCCGAGGG AATTTACATA
      CTGACCGGAC CTATGGTATA AAGCCCGGTC GTCGGCTCCC TTAAATGTAT

3701  GAGGGGCTAA TGCACAATCA AGATGGTTTA ATCTGTGGGT TGAGACAGCT
      CTCCCCGATT ACGTGTTAGT TCTACCAAAT TAGACACCCA ACTCTGTCGA

3751  GGCCAACGAG ACGACTCAAG CTCTTCAACT GTTCCTGAGA GCCACAACTG
      CCGGTTGCTC TGCTGAGTTC GAGAAGTTGA CAAGGACTCT CGGTGTTGAC

3801  AGCTACGCAC CTTTTCAATC CTCAACCGTA AGGCAATTGA TTTCTTGCTG
      TCGATGCGTG GAAAAGTTAG GAGTTGGCAT TCCGTTAACT AAAGAACGAC

3851  CAGCGATGGG GCGGCACATG CCACATTCTG GGACCGGACT GCTGTATCGA
      GTCGCTACCC CGCCGTGTAC GGTGTAAGAC CCTGGCCTGA CGACATAGCT

3901  ACCACATGAT TGGACCAAGA ACATAACAGA CAAAATTGAT CAGATTATTC
      TGGTGTACTA ACCTGGTTCT TGTATTGTCT GTTTTAACTA GTCTAATAAG

3951  ATGATTTTGT TGATAAAACC CTTCCGGACC AGGGGGACAA TGACAATTGG
      TACTAAAACA ACTATTTTGG GAAGGCCTGG TCCCCCTGTT ACTGTTAACC

4001  TGGACAGGAT GGAGACAATG GATACCGGCA GGTATTGGAG TTACAGGCGT
      ACCTGTCCTA CCTCTGTTAC CTATGGCCGT CCATAACCTC AATGTCCGCA

4051  TATAATTGCA GTTATCGCTT TATTCTGTAT ATGCAAATTT GTCTTTTAGT
      ATATTAACGT CAATAGCGAA ATAAGACATA TACGTTTAAA CAGAAAATCA

4101  TTTTCTTCAG ATTGCTTCAT GGAAAAGCTC AGCCTCAAAT CAATGAAACC
      AAAAGAAGTC TAACGAAGTA CCTTTTCGAG TCGGAGTTTA GTTACTTTGG

4151  AGGATTTAAT TATATGGATT ACTTGAATCT AAGATTACTT GACAAATGAT
      TCCTAAATTA ATATACCTAA TGAACTTAGA TTCTAATGAA CTGTTTACTA

4201  AATATAATAC ACTGGAGCTT TAAACATAGC CAATGTGATT CTAACTCCTT
      TTATATTATG TGACCTCGAA ATTTGTATCG GTTACACTAA GATTGAGGAA

4251  TAAACTCACA GTTAATCATA AACAAGGTTT GGAATTGATC TGCTGTGCCT
      ATTTGAGTGT CAATTAGTAT TTGTTCCAAA CCTTAACTAG ACGACACGGA

4301  TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC
      AGATCAACGG TCGGTAGACA ACAAACGGGG AGGGGGCACG GAAGGAACTG

4351  CCTGGAAGGT GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG
      GGACCTTCCA CGGTGAGGGT GACAGGAAAG GATTATTTTA CTCCTTTAAC

4401  CATCGCATTG TCTGAGTAGG TGTCATTCTA TTCTGGGGGG TGGGGTGGGG
      GTAGCGTAAC AGACTCATCC ACAGTAAGAT AAGACCCCCC ACCCCACCCC
```

```
                                                      SphI
4451  CAGCACAGCA AGGGGGAGGA TTGGGAAGAC AATAGCAGGC ATGCTGGGGA
      GTCGTGTCGT TCCCCCTCCT AACCCTTCTG TTATCGTCCG TACGACCCCT

                          KpnI
4501  TGCGGTGGGC TCTATGGGTA CCCAGGTGCT GAAGAATTGA CCCGGTTCCT
      ACGCCACCCG AGATACCCAT GGGTCCACGA CTTCTTAACT GGGCCAAGGA

4551  CCTGGGCCAG AAAGAAGCAG GCACATCCCC TTCTCTGTGA CACACCCTGT
      GGACCCGGTC TTTCTTCGTC CGTGTAGGGG AAGAGACACT GTGTGGGACA

4601  CCACGCCCCT GGTTCTTAGT TCCAGCCCCA CTCATAGGAC ACTCATAGCT
      GGTGCGGGGA CCAAGAATCA AGGTCGGGGT GAGTATCCTG TGAGTATCGA

4651  CAGGAGGGCT CCGCCTTCAA TCCCACCCGC TAAAGTACTT GGAGCGGTCT
      GTCCTCCCGA GGCGGAAGTT AGGGTGGGCG ATTTCATGAA CCTCGCCAGA

4701  CTCCCTCCCT CATCAGCCCA CCAAACCAAA CCTAGCCTCC AAGAGTGGGA
      GAGGGAGGGA GTAGTCGGGT GGTTTGGTTT GGATCGGAGG TTCTCACCCT

4751  AGAAATTAAA GCAAGATAGG CTATTAAGTG CAGAGGGAGA GAAAATGCCT
      TCTTTAATTT CGTTCTATCC GATAATTCAC GTCTCCCTCT CTTTTACGGA

4801  CCAACATGTG AGGAACTAAT GAGAGAAATC ATAGAATTTC TTCCGCTTCC
      GGTTGTACAC TCCTTGATTA CTCTCTTTAG TATCTTAAAG AAGGCGAAGG

4851  TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC
      AGCGAGTGAC TGAGCGACGC GAGCCAGCAA GCCGACGCCG CTCGCCATAG

4901  AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG
      TCGAGTGAGT TTCCGCCATT ATGCCAATAG GTGTCTTAGT CCCCTATTGC

4951  CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA
      GTCCTTTCTT GTACACTCGT TTTCCGGTCG TTTTCCGGTC CTTGGCATTT

5001  AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA
      TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG GACTGCTCGT

5051  TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT
      AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC TGTCCTGATA

5101  AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT
      TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC GAGAGGACAA

5151  CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG
      GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG GAAGCCCTTC

5201  CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG
      GCACCGCGAA AGAGTTACGA GTGCGACATC CATAGAGTCA AGCCACATCC

5251  TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC
      AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA AGTCGGGCTG

5301  CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA
      GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGGTTGG GCCATTCTGT
```

5351 CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA
GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA TCGTCTCGCT

5401 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG ATTGATGCCG

5451 TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC
ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT TCGGTCAATG

5501 CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG
GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT TGGTGGCGAC

5551 GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA
CATCGCCACC AAAAAAACAA ACGTTCGTCG TCTAATGCGC GTCTTTTTTT

5601 GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG
CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC TGCGAGTCAC

5651 GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA
CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT AGTTTTTCCT

5701 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
AGAAGTGGAT CTAGGAAAAT TTAATTTTTA CTTCAAAATT TAGTTAGATT

5751 AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA
TCATATATAC TCATTTGAAC CAGACTGTCA ATGGTTACGA ATTAGTCACT

5801 GGCACCTATC TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC
CCGTGGATAG AGTCGCTAGA CAGATAAAGC AAGTAGGTAT CAACGGACTG

5851 TCCCGGGGGG GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA
AGGGCCCCCC CCCCCGCGAC TCCAGACGGA GCACTTCTTC CACAACGACT

5901 CTCATACCAG GCCTGAATCG CCCCATCATC CAGCCAGAAA GTGAGGGAGC
GAGTATGGTC CGGACTTAGC GGGGTAGTAG GTCGGTCTTT CACTCCCTCG

5951 CACGGTTGAT GAGAGCTTTG TTGTAGGTGG ACCAGTTGGT GATTTTGAAC
GTGCCAACTA CTCTCGAAAC AACATCCACC TGGTCAACCA CTAAAACTTG

6001 TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT GCGTGATCTG
AAAACGAAAC GGTGCCTTGC CAGACGCAAC AGCCCTTCTA CGCACTAGAC

6051 ATCCTTCAAC TCAGCAAAAG TTCGATTTAT TCAACAAAGC CGCCGTCCCG
TAGGAAGTTG AGTCGTTTTC AAGCTAAATA AGTTGTTTCG GCGGCAGGGC

6101 TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA ACCAATTCTG
AGTTCAGTCG CATTACGAGA CGGTCACAAT GTTGGTTAAT TGGTTAAGAC

6151 ATTAGAAAAA CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA
TAATCTTTTT GAGTAGCTCG TAGTTTACTT TGACGTTAAA TAAGTATAGT

6201 GGATTATCAA TACCATATTT TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA
CCTAATAGTT ATGGTATAAA AACTTTTTCG GCAAAGACAT TACTTCCTCT

6251 AAACTCACCG AGGCAGTTCC ATAGGATGGC AAGATCCTGG TATCGGTCTG
TTTGAGTGGC TCCGTCAAGG TATCCTACCG TTCTAGGACC ATAGCCAGAC

```
6301  CGATTCCGAC TCGTCCAACA TCAATACAAC CTATTAATTT CCCCTCGTCA
      GCTAAGGCTG AGCAGGTTGT AGTTATGTTG GATAATTAAA GGGGAGCAGT

6351  AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTCACGA CTGAATCCGG
      TTTTATTCCA ATAGTTCACT CTTTAGTGGT ACTCAGTGCT GACTTAGGCC

                HindIII
6401  TGAGAATGGC AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC
      ACTCTTACCG TTTTCGAATA CGTAAAGAAA GGTCTGAACA AGTTGTCCGG

6451  AGCCATTACG CTCGTCATCA AAATCACTCG CATCAACCAA ACCGTTATTC
      TCGGTAATGC GAGCAGTAGT TTTAGTGAGC GTAGTTGGTT TGGCAATAAG

                                             PvuI
6501  ATTCGTGATT GCGCCTGAGC GAGACGAAAT ACGCGATCGC TGTTAAAAGG
      TAAGCACTAA CGCGGACTCG CTCTGCTTTA TGCGCTAGCG ACAATTTTCC

6551  ACAATTACAA ACAGGAATCG AATGCAACCG GCGCAGGAAC ACTGCCAGCG
      TGTTAATGTT TGTCCTTAGC TTACGTTGGC CGCGTCCTTG TGACGGTCGC

6601  CATCAACAAT ATTTTCACCT GAATCAGGAT ATTCTTCTAA TACCTGGAAT
      GTAGTTGTTA TAAAAGTGGA CTTAGTCCTA TAAGAAGATT ATGGACCTTA

6651  GCTGTTTTCC CGGGGATCGC AGTGGTGAGT AACCATGCAT CATCAGGAGT
      CGACAAAAGG GCCCCTAGCG TCACCACTCA TTGGTACGTA GTAGTCCTCA

6701  ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCC GTCAGCCAGT
      TGCCTATTTT ACGAACTACC AGCCTTCTCC GTATTTAAGG CAGTCGGTCA

6751  TTAGTCTGAC CATCTCATCT GTAACATCAT TGGCAACGCT ACCTTTGCCA
      AATCAGACTG GTAGAGTAGA CATTGTAGTA ACCGTTGCGA TGGAAACGGT

6801  TGTTTCAGAA ACAACTCTGG CGCATCGGGC TTCCCATACA ATCGATAGAT
      ACAAAGTCTT TGTTGAGACC GCGTAGCCCG AAGGGTATGT TAGCTATCTA

6851  TGTCGCACCT GATTGCCCGA CATTATCGCG AGCCCATTTA TACCCATATA
      ACAGCGTGGA CTAACGGGCT GTAATAGCGC TCGGGTAAAT ATGGGTATAT

                                             XhoI
6901  AATCAGCATC CATGTTGGAA TTTAATCGCG GCCTCGAGCA AGACGTTTCC
      TTAGTCGTAG GTACAACCTT AAATTAGCGC CGGAGCTCGT TCTGCAAAGG

6951  CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTACTGTTTA TGTAAGCAGA
      GCAACTTATA CCGAGTATTG TGGGGAACAT AATGACAAAT ACATTCGTCT

7001  CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC
      GTCAAAATAA CAAGTACTAC TATATAAAAA TAGAACACGT TACATTGTAG

                  DraIII
7051  AGAGATTTTG AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC
      TCTCTAAAAC TCTGTGTTGC ACCGAAAGGG GGGGGGGGGT AATAACTTCG

7101  ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA
      TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC TTACATAAAT
```

```
7151  GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC
      CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT TTTCACGGTG

7201  CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG
      GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT ATTTTTATCC

7251  CGTATCACGA GGCCCTTTCG TC
      GCATAGTGCT CCGGGAAAGC AG
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01382

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00; C07H 21/04; C12N 15/63, 15/86, 5/10, 15/40
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 93.2, 93.21; 536/23.1, 23.72; 435/5, 6, 455, 457, 458, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Biosis, Medline, Biotech
Search terms: Ebola virus, glycoprotein, transmembrane, targeting

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,166,320 A (WU et al.) 24 November 1992, columns 9-10 and claims 1-18.	1-20
A	SANCHEZ et al. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proceedings of the National Academy of Sciences. April 1996, Vol. 93, pages 3602-3607, especially page 3604.	3, 12
A	FELGNER et al. Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. Proceedings of the National Academy of Sciences. November 1987, Vol. 84, pages 7413-7417, especially page 7414.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 APRIL 1999	11 MAY 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	DAVID GUZO Lawrence Fox
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VOLCHKOV et al. GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. Virology. 1995, Vol. 214, pages 421-430, especially page 424.	3, 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01382

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/450, 93.2, 93.21; 536/23.1, 23.72; 435/5, 6, 455, 457, 458, 320.1